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(54) Title: NOVEL HUMAN TRANSPORTER PROTEINS			
(57) Abstract			
<p>The present invention provides two novel families of novel human transporter proteins (NTPs). The invention additionally provides for agonists, antagonists, antibodies, antisense molecules that are specific for the NTPs, and further provides genetically engineered expression vectors for the NTPs and host comprising the same. The invention further provides for processes for identifying/producing molecules that effect NTP activity which comprise the use of the disclosed NTPs or genes encoding the same.</p>			

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## NOVEL HUMAN TRANSPORTER PROTEINS

This application claims priority under 35 U.S.C. §119 (e) to U.S. provisional patent application no. 60/130,552 filed April 22, 1999, which is hereby incorporated by reference in its entirety.

### 1. INTRODUCTION

The present invention relates to the discovery, identification and characterization of novel human polynucleotides that encode proteins sharing structural similarity with transporter proteins. The invention encompasses the described polynucleotides, host cell expression systems, the encoded proteins, fusion proteins, polypeptides and peptides, antibodies to the encoded proteins and peptides, and genetically engineered animals that lack at least one of the disclosed genes, or over express the disclosed genes, antagonists and agonists of the described proteins, and other compounds that modulate the expression or activity of the proteins encoded by the disclosed genes that can be used for diagnosis, drug screening, clinical trial monitoring, and/or the treatment of physiological or behavioral disorders.

### 2. BACKGROUND OF THE INVENTION

Transporter proteins are used to facilitate the translocation of certain molecules either into or out of the cell. Often, such transporters work by "pumping" ions across the cell membrane and co-transporting specific molecules (amino acids, amino acid derivatives and precursors, dicarboxylates, inorganic molecules, etc.) across the membrane. Such mechanisms play important roles in maintaining cellular and metabolic homeostasis, neuron function, signaling, and drug resistance. As such, transporter proteins constitute compelling targets for the development and study of novel therapeutic agents.

### 3. SUMMARY OF THE INVENTION

The present invention relates to the discovery, identification and characterization of nucleotides that encode novel transporter proteins, and the corresponding amino acid sequences of the described novel transporter proteins (NTPs). The NTPs described for the first time herein, are transmembrane proteins that span the cellular membrane and are

involved in translocating molecules across membranes. The described NTPs share structural similarity with a variety of transporter proteins. The sequences encoding the NTPs were initially identified via chimeric gene trap transcripts generated in human cells. The novel human NTPs described herein, encode proteins of 627, 627, 581, 627, 627, 581, 5 626, 626, 580, 626, 626, 580, 672, 672, 258, and 258 amino acids in length (see SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32 respectively). The described NTPs have hydrophobic leader sequences and transmembrane regions (of about 20-30 amino acids) characteristic of transporter proteins.

The invention encompasses the nucleotides presented in the Sequence Listing, host 10 cells expressing such nucleotides, the expression products of such nucleotides, and: (a) nucleotides that encode mammalian homologues of the described NTPs including the specifically described human NTP genes; (b) nucleotides that encode one or more portions that correspond to functional domains of a NTP, as well as the polypeptide products specified by such nucleotide sequences, including but not limited to the novel regions of any 15 extracellular domain(s) (ECD), one or more transmembrane domain(s) (TM), and the cytoplasmic domain(s) (CD); (c) isolated nucleotides that encode mutants, engineered or naturally occurring, of the described NTPs in which all or a part of at least one of the domains is deleted or altered, and the polypeptide products specified by such nucleotide sequences, including but not limited to soluble receptors in which all or a portion of a TM is 20 deleted, and nonfunctional receptors in which all or a portion of a CD is deleted; (d) nucleotides that encode fusion proteins containing the coding region from a NTP, or one of its domains (*e.g.*, an extracellular domain) fused to another peptide or polypeptide.

The invention also encompasses agonists and antagonists of the NTPs, including small molecules, large molecules, mutated NTPs, or portions thereof, and antibodies, as 25 well as nucleotide sequences that can be used to inhibit the expression of the described NTPs (*e.g.*, antisense and ribozyme molecules, and gene or regulatory sequence replacement constructs) or to enhance the expression of the described NTP genes (*e.g.*, expression constructs that place the described genes under the control of a strong promoter), and transgenic animals that express a NTP transgene or "knock-outs" (which can be conditional) 30 that do not express a functional NTP (see, for example, PCT Applic. No. PCT/US98/03243, filed February 20, 1998, herein incorporated by reference). In addition to knock-outs, an

additional aspect of the present invention includes animals having genetically engineered mutations in at least one of the described genes that modify the activity or expression of the NTP (*i.e.*, point mutations, over-expression mutations, etc.).

Further, the present invention also relates to methods for the use of the described  
5 NTP genes and/or NTPs for the identification of compounds that modulate, *i.e.*, act as agonists or antagonists, of NTP gene expression and or NTP activity. Such compounds can be used as therapeutic agents for the treatment of various symptomatic representations of biological disorders or imbalances.

#### 4. DESCRIPTION OF THE SEQUENCE LISTING

10 The Sequence Listing provides the sequence of the described NTP polynucleotides, and the amino acid sequences encoded thereby.

#### 5. DETAILED DESCRIPTION OF THE INVENTION

The human NTPs described for the first time herein, are novel transporter proteins that are expressed by genes present in human cells. As transporter proteins, the NTPs are  
15 membrane associated proteins that span the lipid bilayer (*i.e.*, transmembrane proteins). Transporter proteins perform a wide variety of roles in the body varying from, *inter alia*, amino acid scavenging, recycling neurotransmitters, control of blood volume/pressure, nutrient and fluid absorption, energy production, etc. Interfering with, neutralizing, or enhancing NTP function can thus effect a wide variety of physiological changes. Because  
20 of their biological significance, transporter proteins have been subjected to substantial scientific/commercial scrutiny (see, for example, U.S. Application Ser. Nos. 08/805,118 filed February 24, 1997, and 60/047,131 filed May 20, 1997, and U.S. Patent Nos. 5,686,266, 5,882,926, and 5,618,677 which are herein incorporated by reference in their entirety).

25 The invention encompasses the use of the described NTP nucleotides, NTPs, peptides and fusions derived therefrom, as well as antibodies, preferably humanized monoclonal antibodies, or binding fragments, domains, or fusion proteins thereof, to the NTPs (which can, for example, act as NTP agonists or antagonists), antagonists that inhibit NTP activity or expression, or agonists that activate NTP activity or increase NTP

expression, or can be used in the diagnosis and treatment of NTP-related diseases or disorders. Examples of such NTP-related diseases include, but are not limited to, seizures, mental illness, dementia, diabetes, Alzheimer's disease, depression, kidney disease, digestive/bowel disorders, high blood pressure, cardiopulmonary disease, infectious  
5 diseases, arrhythmia, and cancer.

In particular, the invention described in the subsections below encompasses NTPs, NTP polypeptides or peptides corresponding to functional domains of the NTPs (*e.g.*, ECD, TM or CD), mutated, truncated or deleted forms of the NTPs (*e.g.*, modified versions missing one or more functional domains or portions thereof, such as,  $\Delta$ ECD,  $\Delta$ TM and/or  
10  $\Delta$ CD), NTP fusion proteins (*e.g.*, an NTP or a functional domain of an NTP, such as an ECD, fused to an unrelated protein or peptide such as an immunoglobulin constant region, *i.e.*, IgFc), nucleotide sequences encoding such products, and host cell expression systems that can produce such NTP products.

The invention also encompasses antibodies and anti-idiotypic antibodies (including  
15 Fab fragments), antagonists and agonists of the described NTPs, as well as compounds or nucleotide constructs that inhibit the expression of the NTP genes (transcription factor inhibitors, antisense and ribozyme molecules, or gene or regulatory sequence replacement constructs), or promote expression of NTPs (*e.g.*, expression constructs in which NTP coding sequences are operatively associated with expression control elements such as  
20 promoters, promoter/enhancers, etc.). The invention also relates to host cells and animals genetically engineered to express the human NTPs (or mutant variants thereof) or to inhibit or "knock-out" expression of an animal's endogenous NTP gene. Another variation of the such knock-out animals includes "knock-in" animals where the endogenous copy of the animal gene has been replaced by related activity encoded by a sequence that is not native to  
25 the host animal (*e.g.*, where a human ortholog has been used to replace the corresponding endogenous gene).

The described NTPs, or peptides therefrom, NTP fusion proteins, NTP nucleotide sequences, antibodies, antagonists and agonists can be useful for the detection of mutant NTPs or inappropriately expressed variants of the NTP for the diagnosis of NTP-related  
30 diseases or disorders. The NTPs, or peptides therefrom, NTP fusion proteins, NTP gene nucleotide sequences, host cell expression systems, antibodies, antagonists, agonists and

genetically engineered cells and animals can also be used for screening for drugs (or high throughput screening of compound "libraries") effective in the treatment of the symptomatic or phenotypic manifestations of perturbing the normal function of a NTP in the body. The use of engineered host cells and/or animals may offer an advantage in that such systems  
5 allow not only for the identification of compounds that bind to an ECD of an NTP, but can also identify compounds that affect the activity of the NTP.

Finally, the NTP products (especially soluble derivatives such as peptides corresponding to the NTP ECD, or truncated polypeptides lacking one or more TM domains) and fusion protein products (especially NTP-Ig fusion proteins, *i.e.*, fusions of an  
10 NTP, or a domain of an NTP, *e.g.*, ECD,  $\Delta$ TM to an IgFc), antibodies and anti-idiotypic antibodies (including Fab fragments), antagonists, or agonists can be used in the treatment of disease. For example, the administration of an effective amount of soluble NTP ECD,  $\Delta$ TM, or an ECD-IgFc fusion protein or an anti-idiotypic antibody (or its Fab) that mimics the NTP ECD would "mop up" or "neutralize" NTP effector, modulatory, or activator,  
15 ligands, and prevent or reduce transporter expression and/or activity. Nucleotide constructs encoding such NTP products can be used to genetically engineer host cells to express such products *in vivo*; these genetically engineered cells function as "bioreactors" in the body delivering a continuous supply of an NTP, a NTP peptide, soluble ECD or  $\Delta$ TM or a NTP fusion protein that can either enhance NTP activity or inhibit NTP activity. Nucleotide  
20 constructs encoding a functional NTP, mutant NTP variants, as well as antisense and ribozyme molecules can thus be used in "gene therapy" approaches for the modulation of NTP expression. Thus, the invention also encompasses pharmaceutical formulations and methods for treating biological disorders that comprise one or more of the described NTP genes or products. Suitable vectors/systems for such gene therapy or gene delivery  
25 applications include, but are not limited to, retrovirus, lentivirus, SIV, HIV, adenovirus, adeno-associated virus, lipid associated (and particularly cationic lipid associated) polynucleotide preparations, micro carrier beads or lattices, herpes virus vectors, hepatitis virus vectors, polynucleotide-containing emulsions, formulations containing "naked" DNA, etc.

30 Various aspects of the invention are described in greater detail in the subsections below.

### 5.1 THE NTP GENES

The cDNA sequences and deduced amino acid sequences of the presently described human NTPs are presented in the Sequence Listing. SEQ ID NOS: 1-24 describe variants of a novel human transporter protein that is expressed in placenta, brain, cerebellum, spinal cord, testis, prostate. Northern analysis reveals a major transcript of approximately 3,2 kb and a minor transcript of about 5 kb in length. cDNAs used for sequencing were isolated from a testis cDNA library. Homology studies using SEQ ID NOS: 1-24 indicated that the described molecules share substantial similarity with, *inter alia*, sodium-dependent sulphate cotransporter proteins and sodium/dicarboxylates cotransporter proteins.

SEQ ID NOS: 25-32 describe variants of a novel human transporter protein that is expressed in brain, cerebellum, spinal cord, adrenal gland, pituitary, testis, stomach, and small intestine. Northern analysis reveals a major transcript (in brain and spinal cord) of approximately 10 kb and possible minor transcripts of about 3.1 and 5 kb in length. cDNAs used for sequencing were isolated from a kidney cDNA library.

Homology studies using SEQ ID NOS: 25-32 indicated that the described molecules share substantial similarity with, *inter alia*, amino acid transporter proteins and particularly cationic amino acid transporter proteins.

The NTPs of the present invention include: (a) the human DNA sequences presented in the Sequence Listing and additionally contemplate any nucleotide sequences encoding a contiguous and functional NTP open reading frame (ORF) that hybridizes to a complement of a DNA sequence presented in the Sequence Listing under highly stringent conditions, *e.g.*, hybridization to filter-bound DNA in 0.5 M NaHPO<sub>4</sub>, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C (Ausubel F.M. *et al.*, eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing

Associates, Inc., and John Wiley & sons, Inc., New York, at p. 2.10.3) and encodes a functionally equivalent gene product. Additionally contemplated are any nucleotide sequences that hybridize to the complement of the DNA sequence that encodes and expresses an amino acid sequence presented in the Sequence Listing under moderately stringent conditions, *e.g.*, washing in 0.2xSSC/0.1% SDS at 42°C (Ausubel *et al.*, 1989, *supra*), yet which still encode a functionally equivalent NTP product. Functional equivalents of NTPs include naturally occurring NTPs present in other species, and mutant

NTPs whether naturally occurring or engineered. The invention also includes degenerate variants of the disclosed sequence.

The invention also includes nucleic acid molecules, preferably DNA molecules, that hybridize to, and are therefore the complements of, the described NTP gene sequences.

- 5 Such hybridization conditions may be highly stringent or less highly stringent, as described above. In instances wherein the nucleic acid molecules are deoxyoligonucleotides ("DNA oligos"), such molecules are typically about 16 to about 100 bases long, about 20 to about 80 bases long or about 34 to about 45 bases long, or any variation or combination of sizes represented therein and which incorporate a contiguous region of sequence first disclosed in
- 10 the Sequence Listing. The described oligonucleotides can be used in conjunction with the polymerase chain reaction (PCR) to screen libraries, isolate clones, and prepare cloning and sequencing templates, etc.. Alternatively, the oligonucleotides can be used as hybridization probes. For oligonucleotide probes, highly stringent conditions may refer, *e.g.*, to washing in 6xSSC/0.05% sodium pyrophosphate at 37°C (for 14-base oligos), 48° C (for 17-base
- 15 oligos), 55° C (for 20-base oligos), and 60° C (for 23-base oligos). These nucleic acid molecules may encode or act as NTP antisense molecules, useful, for example, in NTP gene regulation (and/or as antisense primers in amplification reactions of NTP gene nucleic acid sequences). With respect to NTP gene regulation, such techniques can be used to regulate the biological functions affected by the described NTPs. Further, such sequences may be
- 20 used as part of ribozyme and/or triple helix sequences that are also useful for NTP gene regulation.

- Low stringency conditions are well known to those of skill in the art, and will vary predictably depending on the specific organisms from which the library and the labeled sequences are derived. For guidance regarding such conditions see, for example, Sambrook
- 25 *et al.*, 1989, *Molecular Cloning, A Laboratory Manual* (and periodic updates thereof), Cold Springs Harbor Press, N.Y.; and Ausubel *et al.*, 1989, *Current Protocols in Molecular Biology*, Green Publishing Associates and Wiley Interscience, N.Y.

- Alternatively, suitably labeled NTP nucleotide probes can be used to screen a human genomic library using appropriately stringent conditions or by PCR. The identification and
- 30 characterization of human genomic clones is helpful for identifying polymorphisms, determining the genomic structure of a given locus/allele, and designing diagnostic tests.

For example, sequences derived from regions adjacent to the intron/exon boundaries of the human gene can be used to design primers for use in amplification assays to detect mutations within the exons, introns, splice sites (*e.g.*, splice acceptor and/or donor sites), etc., that can be used in diagnostics and pharmacogenomics.

5 Further, a NTP gene homolog may be isolated from nucleic acid from the organism of interest by performing PCR using two degenerate oligonucleotide primer pools designed on the basis of amino acid sequences within a NTP product disclosed herein. The template for the reaction may be total RNA, mRNA, and/or cDNA obtained by reverse transcription of mRNA prepared from, for example, human or non-human cell lines or tissue that are  
10 known or suspected to express a NTP gene.

The PCR product can be subcloned and sequenced to ensure that the amplified sequences represent the sequence of the desired NTP gene. The PCR fragment may then be used to isolate a full length cDNA clone by a variety of methods. For example, the amplified fragment may be labeled and used to screen a cDNA library such as a  
15 bacteriophage cDNA library. Alternatively, the labeled fragment may be used to isolate genomic clones via the screening of a genomic library.

PCR technology may also be utilized to isolate full length cDNA sequences. For example, RNA may be isolated, following standard procedures, from an appropriate cellular or tissue source (*i.e.*, one known, or suspected, to express a NTP gene, such as, for example,  
20 brain tissue). A reverse transcription (RT) reaction may be performed on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified fragment for the priming of first strand synthesis. The resulting RNA/DNA hybrid may then be "tailed" using a standard terminal transferase reaction, the hybrid may be digested with RNase H, and second strand synthesis may then be primed with a complementary primer. Thus, cDNA  
25 sequences upstream of the amplified fragment may easily be isolated. For a review of cloning strategies which may be used, see *e.g.*, Sambrook *et al.*, 1989, *supra*.

A cDNA of a mutant NTP gene may be isolated, for example, by using PCR. In this case, the first cDNA strand may be synthesized by hybridizing an oligo-dT oligonucleotide to mRNA isolated from tissue known or suspected to be expressed in an individual  
30 putatively carrying a mutant NTP allele, and by extending the new strand with reverse transcriptase. The second strand of the cDNA is then synthesized using an oligonucleotide

that hybridizes specifically to the 5' end of the normal gene. Using these two primers, the product is then amplified via PCR, optionally cloned into a suitable vector, and subjected to DNA sequence analysis through methods well known to those of skill in the art. By comparing the DNA sequence of the mutant NTP allele to that of a corresponding normal NTP allele, the mutation(s) responsible for the loss or alteration of function of the mutant NTP product can be ascertained.

Alternatively, a genomic library can be constructed using DNA obtained from an individual suspected of or known to carry a mutant NTP allele, or a cDNA library can be constructed using RNA from a tissue known, or suspected, to express a mutant or alternatively spliced NTP allele. A normal NTP gene, or any suitable fragment thereof, can then be labeled and used as a probe to identify the corresponding mutant NTP allele in such libraries. Clones containing the mutant NTP gene sequences can then be purified and subjected to sequence analysis according to methods well known to those of skill in the art.

Additionally, an expression library can be constructed utilizing cDNA synthesized from, for example, RNA isolated from a tissue known, or suspected, to express a mutant NTP allele in an individual suspected of or known to carry such a mutant allele. In this manner, gene products made by the putatively mutant tissue may be expressed and screened using standard antibody screening techniques in conjunction with antibodies raised against a normal NTP product, as described, below, in Section 5.3. (For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.)

Additionally, screening can be accomplished using labeled NTP fusion proteins, such as, for example, AP-NTP or NTP-AP fusion proteins. In cases where a NTP mutation results in an expressed gene product with altered function (e.g., as a result of a missense or a frameshift mutation), a polyclonal set of antibodies to a given NTP are likely to cross-react with the corresponding mutant NTP gene product. Library clones detected by their reaction with such labeled antibodies can be purified and subjected to sequence analysis according to methods well known to those of skill in the art.

The invention also encompasses nucleotide sequences that encode mutant NTPs, peptide fragments of a NTP, truncated NTPs, and NTP fusion proteins. These include, but are not limited to nucleotide sequences encoding mutant NTPs described in section 5.2

*infra*; polypeptides or peptides corresponding to one or more ECD, TM and/or CD domains of a NTP or any portions of such domains; truncated NTPs in which one or more of the domains are deleted, *e.g.*, a soluble NTP lacking TM or both the TM and CD regions, or a truncated, nonfunctional NTP lacking all or a portion of, for example, a CD region.

- 5 Nucleotides encoding fusion proteins may include, but are not limited to, full length NTP sequences, truncated NTPs, or nucleotides encoding peptide fragments of a NTP fused to an unrelated protein or peptide, such as for example, a transmembrane sequence, which anchors a NTP ECD to the cell membrane; an Ig Fc domain which increases the stability and half life of the resulting fusion protein (*e.g.*, NTP-Ig) in the bloodstream; or an enzyme,  
10 fluorescent protein, luminescent protein which can be used as a marker.

- The invention also encompasses (a) DNA vectors that contain any of the NTP coding sequence and/or the complements thereof (*i.e.*, antisense); (b) DNA expression vectors that contain any portion of a NTP coding sequence operatively associated with a regulatory element that directs the expression of the coding sequence; and (c) genetically  
15 engineered host cells engineered to contain NTP coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences in the host cell. As used herein, regulatory elements include, but are not limited to, inducible and non-inducible promoters, enhancers, operators and other elements known to those skilled in the art that drive and regulate expression. Such regulatory elements include, but are not limited  
20 to, the cytomegalovirus hCMV immediate early gene, regulatable, viral (particularly retroviral LTR promoters) the early or late promoters of SV40 adenovirus, the *lac* system, the *trp* system, the *tac* system, the *trc* system, the major operator and promoter regions of phage lambda, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase (PGK), the promoters of acid phosphatase, and the promoters of the yeast  $\alpha$ -mating  
25 factors.

## 5.2 NTP PRODUCTS

- NTPs, peptide fragments therefrom, mutated, truncated or deleted forms of NTPs and/or NTP fusion proteins can be prepared for a variety of uses, including but not limited to the generation of antibodies, as reagents in diagnostic assays, the identification of other  
30 cellular gene products related to, or that interact with, a NTP, as reagents in assays for

screening for compounds that can be as pharmaceutical reagents useful in the therapeutic treatment of mental, biological, or medical disorders and diseases.

The Sequence Listing discloses the amino acid sequences encoded by the described NTP genes. The described NTPs have an initiator methionine in a DNA sequence context  
5 consistent with a translation initiation site, followed by a initiator codon.

The NTP sequences of the present invention include the nucleotide and amino acid sequences presented in the Sequence Listing as well as analogues and derivatives thereof. Further, corresponding NTP homologues from other species are encompassed by the invention. In fact, any NTP protein encoded by the NTP nucleotide sequences described in  
10 Section 5.1, above, are within the scope of the invention, as are any novel polynucleotide sequences encoding all or any novel portion of an amino acid sequence presented in the Sequence Listing. The degenerate nature of the genetic code is well known, and, accordingly, each amino acid presented in the Sequence Listing, is generically representative of the well known nucleic acid "triplet" codon, or in many cases codons, that  
15 can encode the amino acid. As such, as contemplated herein, the amino acid sequence presented in the Sequence Listing, when taken together with the genetic code (see, for example, Table 4-1 at page 109 of "Molecular Cell Biology", 1986, J. Darnell *et al.* eds., Scientific American Books, New York, NY, herein incorporated by reference) are generically representative of all the various permutations and combinations of nucleic acid  
20 sequences that can encode such amino acid sequence (as well as such variants as biased by human codon usage frequency tables).

The invention also encompasses proteins that are functionally equivalent to a NTP encoded by the nucleotide sequence described in Section 5.1, as judged by any of a number of criteria, including but not limited to the ability to bind or transport a ligand of the NTP,  
25 the ability to effect an identical or complementary biological pathway, a change in cellular metabolism (*e.g.*, ion flux, tyrosine phosphorylation, etc.), or to effect the same change in phenotype when the NTP equivalent is present in an appropriate cell type (such as the amelioration, prevention or delay of a biochemical, biophysical, or overt phenotype). Such functionally equivalent NTPs include but are not limited to additions or substitutions of  
30 amino acid residues within the amino acid sequence encoded by a NTP gene sequence described above, in Section 5.1, but which result in a silent change, thus producing a

functionally equivalent gene product. Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

While random mutations can be made to NTP DNA (using random mutagenesis techniques well known to those skilled in the art) and the resulting mutant NTPs tested for activity, site-directed mutations of a NTP coding sequence can be engineered (using site-directed mutagenesis techniques well known to those skilled in the art) to generate mutant NTPs with increased function, *e.g.*, higher binding/transport affinity for the transporter substrate, or decreased function. One starting point for such analysis is by aligning the disclosed human sequences with corresponding gene/protein sequences from, for example, other mammals in order to identify amino acid sequences and motifs that are conserved between different species. Non-conservative changes can be engineered at variable positions to alter function, transporter activity, or both. Alternatively, where alteration of function is desired, deletion or non-conservative alterations of the conserved regions (*i.e.*, identical amino acids) can be engineered. For example, deletion or non-conservative alterations (substitutions or insertions) of the various conserved transmembrane domains.

Other mutations to a NTP coding sequence can be made to generate NTPs that are better suited for expression, scale up, etc. in the host cells chosen. For example, cysteine residues can be deleted or substituted with another amino acid in order to eliminate disulfide bridges; N-linked glycosylation sites can be altered or eliminated to achieve, for example, expression of a homogeneous product that is more easily recovered and purified from yeast hosts which are known to hyperglycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first or third amino acid positions of any one or more of the glycosylation recognition sequences which occur in an ECD (N-X-S or N-X-T), and/or an amino acid deletion at the second position of any one or more such recognition

sequences in an ECD will prevent glycosylation of the NTP at the modified tripeptide sequence. (See, *e.g.*, Miyajima *et al.*, 1986, EMBO J. 5(6):1193-1197).

Peptides corresponding to one or more domains of a NTP (*e.g.*, ECD, TM, CD, etc.), truncated or deleted NTPs (*e.g.*, a NTP in which a ECD, TM and/or CD, or any portion thereof, is deleted) as well as fusion proteins in which a full length NTP, NTP peptide, or truncated NTP is fused to an unrelated protein, are also within the scope of the invention, and can be designed on the basis of the presently disclosed NTP gene nucleotide and NTP amino acid sequences. Such fusion proteins include but are not limited to IgFc fusions which stabilize a NTP or peptide and prolong half-life *in vivo*; or fusions to any amino acid sequence that allows the fusion protein to be anchored to the cell membrane, allowing an ECD to be exhibited on the cell surface; or fusions to an enzyme, fluorescent protein, or luminescent protein which provide a marker function.

While a NTP, and corresponding peptides, can be chemically synthesized (*e.g.*, see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y.), large polypeptides derived from a NTP and especially a full-length NTP product can be advantageously produced by recombinant DNA technology using techniques well known in the art for expressing proteins. Such methods can be used to construct expression vectors containing a NTP nucleotide sequence described in Section 5.1 and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. See, for example, the techniques described in Sambrook *et al.*, 1989, *supra*, and Ausubel *et al.*, 1989, *supra*. Alternatively, RNA corresponding to all or a portion of a transcript encoded by a NTP gene sequence may be chemically synthesized using, for example, synthesizers. See, for example, the techniques described in "Oligonucleotide Synthesis", 1984, Gait, M.J. ed., IRL Press, Oxford, which is incorporated by reference herein in its entirety.

A variety of host-expression vector systems may be utilized to express a NTP gene nucleotide sequence of the invention. Where the NTP peptide or polypeptide is a soluble derivative (*e.g.*, NTP peptides corresponding to an ECD; truncated or deleted NTP in which a TM and/or CD are deleted) the peptide or polypeptide can be recovered from the culture, *i.e.*, from the host cell in cases where the NTP peptide or polypeptide is not secreted, and

from the culture media in cases where the NTP peptide or polypeptide is secreted by the cells. However, such expression systems also encompass engineered host cells that express NTP, or a functional equivalent, *in situ*, *i.e.*, anchored in the cell membrane. Purification or enrichment of NTP from such expression systems can be accomplished using appropriate  
5 detergents and lipid micelles and methods well known to those skilled in the art. However, such engineered host cells themselves may be used in situations where it is important not only to retain the structural and functional characteristics of a NTP, but to assess biological activity, *e.g.*, in drug screening assays.

The expression systems that can be used for purposes of the invention include but  
10 are not limited to microorganisms such as bacteria (*e.g.*, *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing the NTP nucleotide sequence; yeast (*e.g.*, *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing a NTP nucleotide sequence; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculo virus)  
15 containing a NTP sequence; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing a NTP nucleotide sequence; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3, etc.) harboring recombinant expression constructs containing promoters derived from the genome of  
20 mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the NTP product to be expressed. For example, when a large quantity of such a protein is to be produced for the generation of pharmaceutical  
25 compositions comprising a NTP, or for raising antibodies to a NTP, or corresponding peptide, for example, vectors that direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther *et al.*, 1983, EMBO J. 2:1791), in which a NTP gene coding sequence may be ligated into the vector in frame with the *lacZ*  
30 coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 264:5503-

5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The PGEX vectors are  
5 designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhidrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. A NTP gene coding sequence may be cloned individually into non-essential regions (for  
10 example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of NTP gene coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (*i.e.*, virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in  
15 which the inserted gene is expressed (*e.g.*, see Smith *et al.*, 1983, J. Virol. 46: 584; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, a nucleotide sequence from a NTP gene can be ligated to an adenovirus transcription/translation control  
20 complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene can then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the NTP product in infected host cells (*e.g.*, See Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:3655-3659). Specific initiation  
25 signals can also be required for efficient translation of inserted NTP gene nucleotide sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire NTP gene or cDNA, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of a NTP coding  
30 sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with

the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can have a variety of origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (See Bittner *et al.*, 1987, *Methods in Enzymol.* 153:516-544).

In addition, a host cell strain can be chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include, but are not limited to, CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, and WI38 cell lines.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express a NTP can be engineered. Rather than using expression vectors that contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer sequences; transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines that express a NTP product. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that affect the endogenous activity of a NTP.

A number of selection systems can be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, *et al.*, 1977, *Cell* 11:223), hypoxanthine-guanine

phosphoribosyltransferase (Szybalski & Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (Lowy, *et al.*, 1980, Cell 22:817) genes can be employed in tk<sup>-</sup>, hgp<sup>+</sup> or ap<sup>+</sup> cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler, *et al.*, 1980, Natl. Acad. Sci. USA 77:3567; O'Hare, *et al.*, 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, *et al.*, 1981, J. Mol. Biol. 150:1); and hyg<sup>+</sup>, which confers resistance to hygromycin (Santerre, *et al.*, 1984, Gene 30:147).

Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht *et al.* allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht, *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88: 8972-8976). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni<sup>2+</sup>-nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

NTP product(s) can also be expressed in transgenic animals. Animals of any species, including, but not limited to, worms, mice, rats, rabbits, guinea pigs, pigs, micro-pigs, birds, goats, dogs, cats, and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees may be used to generate NTP transgenic animals.

Any technique known in the art may be used to introduce a NTP transgene into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to pronuclear microinjection (Hoppe, P.C. and Wagner, T.E., 1989, U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into germ line cells (Van der Putten *et al.*, 1985, Proc. Natl. Acad. Sci., USA 82:6148-6152); gene targeting in embryonic stem cells (Thompson *et al.*, 1989, Cell 56:313-321); electroporation of embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814); and sperm-mediated gene transfer (Lavitrano *et al.*, 1989, Cell 57:717-723); etc. For a review of such techniques, see Gordon, 1989, Transgenic Animals, Intl. Rev. Cytol. 115:171-229, which is incorporated by reference herein in its entirety.

The present invention provides for transgenic animals that carry the NTP TRANSGENE in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals or somatic cell transgenic animals. The transgene may be integrated as a single transgene or in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:6232-6236. The regulatory sequences required for such cell type-specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

When it is desired that the NTP transgene be integrated into the chromosomal site of the endogenous NTP gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to an endogenous NTP gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, a mutagenic sequence into the targeted NTP gene that effectively disrupts the function of the endogenous gene (*i.e.*, "knockout" cells and animals).

The transgene may also be selectively introduced into a particular cell type, thus inactivating an endogenous NTP gene in only that cell type, by following, for example, the teaching of Gu *et al.*, 1994, Science, 265:103-106. The regulatory sequences required for such cell type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of a recombinant NTP gene can be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR to analyze animal tissues to determine whether the transgene has integrated into the genome. The level of mRNA expression by the transgene in the tissues of the transgenic animals can also be assessed using techniques which include but are not limited to Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and RT-PCR. Samples of NTP gene-expressing tissue, can also be evaluated immunocytochemically using antibodies specific for the NTP product of the transgene.

### 5.3 ANTIBODIES TO NTP PROTEINS

Antibodies that specifically recognize one or more epitopes of a NTP, or epitopes of conserved variants of a NTP, or peptide fragments of a NTP are also encompassed by the invention. Such antibodies include but are not limited to polyclonal antibodies, monoclonal  
5 antibodies (mAbs), human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')<sub>2</sub> fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above.

The antibodies of the invention can be used, for example, in the detection of a NTP in a biological sample and can therefore be utilized as part of a diagnostic or prognostic  
10 technique whereby patients are tested for abnormal amounts of NTP expression or activity. Such antibodies may also be utilized in conjunction with, for example, compound screening schemes, as described, below, in Section 5.5, for the evaluation of the effect of test compounds on the expression and/or activity of a NTP product. Additionally, such antibodies can be used in conjunction with gene therapy to, for example, evaluate the  
15 expression of normal and/or engineered NTP by cells prior to their introduction into the patient. Such antibodies may additionally be used as a method for the inhibition of abnormal NTP activity. Thus, such antibodies can be utilized as part of treatment methods for NTP-involved biological disorders.

For the production of antibodies, various host animals can be immunized by injection  
20 with the NTP, a NTP peptide (e.g., one corresponding to a functional domain of the receptor, such as an ECD, TM or CD), truncated NTP polypeptides (NTP in which one or more domains, e.g., a TM or CD has been deleted, or a portion thereof), functional equivalents of the NTP or NTP mutants. Such host animals may include, but are not limited to, rabbits, goats, mice, and rats, to name but a few. Various adjuvants may be used to  
25 increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Polyclonal  
30 antibodies are heterogeneous populations of antibody molecules derived from the sera of the immunized animals.

Monoclonal antibodies are homogeneous populations of antibodies that bind a particular antigen, and can be obtained by any technique which provides for the production of antibody molecules by, for example, cell lines in culture. These include, but are not limited to, the hybridoma technique of Kohler and Milstein, (1975, *Nature* 256:495-497; and  
5 U.S. Patent No. 4,376,110), the human B-cell hybridoma technique (Kosbor *et al.*, 1983, *Immunology Today* 4:72; Cole *et al.*, 1983, *Proc. Natl. Acad. Sci. USA* 80:2026-2030), and the EBV-hybridoma technique (Cole *et al.*, 1985, *Monoclonal Antibodies And Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma  
10 producing the mAb of this invention can be cultivated in vitro or in vivo. Production of high titers of mAbs in vivo makes this the presently preferred method of production.

Additionally, recombinant antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. A chimeric  
15 antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region. (See, *e.g.*, Cabilly *et al.*, U.S. Patent No. 4,816,567; and Boss *et al.*, U.S. Patent No. 4,816,397, which are incorporated herein by reference in their entirety.) Humanized antibodies are antibody molecules from non-human species having  
20 one or more complementarily determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule. (See, *e.g.*, Queen, U.S. Patent No. 5,585,089, which is incorporated herein by reference in its entirety.) Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT Publication No. WO 87/02671;  
25 European Patent Application 184,187; European Patent Application 171,496; European Patent Application 173,494; PCT Publication No. WO 86/01533; U.S. Patent No. 4,816,567; European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *Bio/Techniques* 4:214; U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature*

321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Such antibodies can be produced, for example, using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, *e.g.*, all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, *see, e.g.*, U.S. Patent 5,625,126; U.S. Patent 5,633,425; U.S. Patent 5,569,825; U.S. Patent 5,661,016; and U.S. Patent 5,545,806. In addition, companies such as Abgenix, Inc. (Fremont, CA), can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, *e.g.*, a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers *et al.* (1994) *Bio/technology* 12:899-903).

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778; Bird, 1988, *Science* 242:423-426; Huston *et al.*, 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward *et al.*, 1989, *Nature* 334:544-546) can be adapted to produce single chain antibodies against NTP products. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')<sub>2</sub>

fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')<sub>2</sub> fragments. Alternatively, Fab expression libraries may be constructed (Huse *et al.*, 1989, Science, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the  
5 desired specificity.

Antibodies to NTP can be utilized to generate anti-idiotypic antibodies that "mimic" NTP, using techniques well known to those skilled in the art. (See, *e.g.*, Greenspan & Bona, 1993, FASEB J 7(5):437-444; and Nissinoff, 1991, J. Immunol. 147(8):2429-2438). For example antibodies which bind to a NTP ECD and competitively inhibit the binding of a  
10 ligand or accessory molecule of a NTP and can thus be used to generate anti-idiotypes that "mimic" a NTP ECD and, therefore, bind and neutralize a ligand or a NTP accessory molecule. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypic antibodies can be used in therapeutic regimens involving the regulation of NTP activity..

#### 5.4 DIAGNOSIS OF ABNORMALITIES RELATED TO A NTP

15 A variety of methods can be employed for the diagnostic and prognostic evaluation of disorders related to NTP function, and for the identification of subjects having a predisposition to such disorders.

Such methods can, for example, utilize reagents such as a NTP nucleotide sequence described in Section 5.1, or a portion thereof, or NTP antibodies as described in Section 5.3.  
20 Specifically, such reagents can be used, for example, for: (1) the detection of the presence of NTP gene mutations, or the detection of either the over- or under-expression of NTP mRNA relative to a given phenotype; (2) the detection of either an over- or under-abundance of a NTP relative to a given phenotype; and (3) the detection of perturbations or abnormalities in the transporter function mediate by a NTP.

25 The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one specific NTP nucleotide sequence or NTP antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings, to diagnose patients exhibiting biological abnormalities.

For the detection of NTP mutations, any nucleated cell can be used as a starting  
30 source for genomic nucleic acid. For the detection of NTP gene expression or NTP gene

products, any cell type or tissue in which a NTP gene is expressed, such as, for example, brain cells, may be utilized.

Nucleic acid-based detection techniques are described, below, in Section 5.4.1.

Peptide detection techniques are described, below, in Section 5.4.2.

#### 5 **5.4.1 DETECTION OF NTP GENES AND TRANSCRIPTS**

Mutations within a NTP gene can be detected using a number of techniques. Nucleic acid from any nucleated cell can be used as the starting point for such assay techniques, and may be isolated according to standard nucleic acid preparation procedures which are well known to those of skill in the art.

10 DNA can be used in hybridization or amplification assays of biological samples to detect abnormalities involving NTP gene structure, including point mutations, insertions, deletions and chromosomal rearrangements. Such assays may include, but are not limited to, Southern analyses, single stranded conformational polymorphism analyses (SSCP), and PCR analyses.

15 Such diagnostic methods for the detection of NTP gene specific mutations can involve for example, contacting and incubating nucleic acids including recombinant DNA molecules, cloned genes or degenerate variants thereof, obtained from a sample, *e.g.*, derived from a patient sample or other appropriate cellular source, with one or more labeled nucleic acid reagents including recombinant DNA molecules, cloned genes or degenerate  
20 variants thereof, as described in Section 5.1, under conditions favorable for the specific annealing of these reagents to their complementary sequences within the NTP gene. Preferably, the lengths of these nucleic acid reagents are at least 15 to 30 nucleotides. After incubation, all non-annealed nucleic acids are removed from the nucleic acid:NTP gene hybrid. The presence of nucleic acids which have hybridized, if any such molecules exist, is  
25 then detected. Using such a detection scheme, the nucleic acid from the cell type or tissue of interest can be immobilized, for example, to a solid support such as a membrane, gene "chip" substrate, or a plastic surface such as that on a microtiter plate or polystyrene beads. In this case, after incubation, non-annealed, labeled nucleic acid reagents of the type described in Section 5.1 are easily removed. Detection of the remaining, annealed, labeled  
30 NTP nucleic acid reagents is accomplished using standard techniques well-known to those in

the art. The NTP gene sequence(s) to which the nucleic acid reagents have annealed can be compared to the annealing pattern expected from a normal NTP gene sequence in order to determine whether a NTP gene mutation is present.

Alternative diagnostic methods for the detection of NTP gene specific nucleic acid molecules, in patient samples or other appropriate cell sources, may involve their  
5 amplification, *e.g.*, by PCR (the experimental embodiment set forth in Mullis, K.B., 1987, U.S. Patent No. 4,683,202), followed by the detection of the amplified molecules using techniques well known to those of skill in the art. The resulting amplified sequences can be compared to those which would be expected if the nucleic acid being amplified contained  
10 only normal copies of a NTP gene in order to determine whether a NTP gene mutation exists.

Additionally, well-known genotyping techniques can be performed to identify individuals carrying NTP gene mutations. Such techniques include, for example, the use of restriction fragment length polymorphisms (RFLPs), which involve sequence variations in  
15 one of the recognition sites for the specific restriction enzyme used.

Additionally, improved methods for analyzing DNA polymorphisms that can be utilized for the identification of NTP gene mutations have been described that capitalize on the presence of variable numbers of short, tandemly repeated DNA sequences between the restriction enzyme sites. For example, Weber (U.S. Pat. No. 5,075,217, which is  
20 incorporated herein by reference in its entirety) describes a DNA marker based on length polymorphisms in blocks of (dC-dA)<sub>n</sub>-(dG-dT)<sub>n</sub> short tandem repeats. The average separation of (dC-dA)<sub>n</sub>-(dG-dT)<sub>n</sub> blocks is estimated to be 30,000-60,000 bp. Markers which are so closely spaced exhibit a high frequency co-inheritance, and are extremely useful in the identification of genetic mutations, such as, for example, mutations within the  
25 NTP gene, and the diagnosis of diseases and disorders related to such NTP mutations.

Also, Caskey *et al.* (U.S. Pat. No. 5,364,759, which is incorporated herein by reference in its entirety) describe a DNA profiling assay for detecting short tri and tetra nucleotide repeat sequences. The process includes extracting the DNA of interest, such as the NTP gene, amplifying the extracted DNA, and labeling the repeat sequences to form a  
30 genotypic map of the individual's DNA.

The level of NTP gene expression can also be assayed by detecting and measuring NTP transcription. For example, RNA from a cell type or tissue known, or suspected to express a NTP gene, such as brain, may be isolated and tested utilizing hybridization or PCR techniques such as are described, above. The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may be a necessary step in the assessment of cells to be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of a NTP gene. Such analyses may reveal both quantitative and qualitative aspects of the expression pattern of a NTP gene, including activation or inactivation of NTP gene expression.

In one embodiment of such a detection scheme, cDNAs are synthesized from the RNAs of interest (*e.g.*, by reverse transcription of the RNA molecule into cDNA). A sequence within the cDNA is then used as the template for a nucleic acid amplification reaction, such as a PCR amplification reaction, or the like. The nucleic acid reagents used as synthesis initiation reagents (*e.g.*, primers) in the reverse transcription and nucleic acid amplification steps of this method are chosen from among the NTP gene nucleic acid reagents described in Section 5.1. The preferred lengths of such nucleic acid reagents are at least 9-30 nucleotides. For detection of the amplified product, the nucleic acid amplification may be performed using radioactively or non-radioactively labeled nucleotides. Alternatively, enough amplified product may be made such that the product may be visualized by standard ethidium bromide staining, by utilizing any other suitable nucleic acid staining method, or by sequencing.

Additionally, it is possible to perform such NTP gene expression assays "in situ", *i.e.*, directly upon tissue sections (fixed and/or frozen) of patient tissue obtained from biopsies or resections, such that no nucleic acid purification is necessary. Nucleic acid reagents such as those described in Section 5.1 may be used as probes and/or primers for such in situ procedures (See, for example, Nuovo, G.J., 1992, "PCR In Situ Hybridization: Protocols And Applications", Raven Press, NY).

Alternatively, if a sufficient quantity of the appropriate cells can be obtained, standard Northern analysis can be performed to determine the level of mRNA expression of a NTP gene.

### 5.4.2 DETECTION OF NTP PRODUCTS

Antibodies directed against wild type or mutant NTPs or variants or peptide fragments thereof, that are discussed, above in Section 5.3, can also be used as diagnostics and prognostics, as described herein. Such diagnostic methods, can be used to detect abnormalities in the level of NTP gene expression, or abnormalities in the structure and/or temporal, tissue, cellular, or subcellular location of a NTP, and can be performed *in vivo* or *in vitro*, such as, for example, on biopsy tissue.

Additionally, antibodies directed to epitopes of a NTP ECD can be used *in vivo* to detect the pattern and level of expression of a NTP in the body. Such antibodies can be labeled, *e.g.*, with a radio-opaque or other appropriate compound and injected into a subject in order to visualize binding to a NTP expressed in the body using methods such as X-rays, CAT-scans, or MRI. Labeled antibody fragments, *e.g.*, the Fab or single chain antibody comprising the smallest portion of the antigen binding region, are preferred for this purpose to promote crossing the blood-brain barrier and permit labeling of NTPs expressed in the brain.

Additionally, any NTP fusion protein or NTP conjugated protein whose presence can be detected, can be administered. For example, NTP fusion or conjugated proteins labeled with a radio-opaque or other appropriate compound can be administered and visualized *in vivo*, as discussed, above for labeled antibodies. Further, such NTP fusion proteins as AP-NTP or NTP-AP fusion proteins can be utilized for *in vitro* diagnostic procedures.

Alternatively, immunoassays or fusion protein detection assays, as described above, can be utilized on biopsy and autopsy samples *in vitro* to permit assessment of the expression pattern of a NTP. Such assays are not confined to the use of antibodies that define a NTP ECD, but can include the use of antibodies directed to epitopes of any of the domains of a NTP, *e.g.*, a ECD, a TM and/or CD. The use of each or all of these labeled antibodies will yield useful information regarding translation and intracellular transport of a NTP to the cell surface, and can identify defects in processing.

The tissue or cell type to be analyzed will generally include those which are known, or suspected, to express a NTP gene, such as, for example, brain cells. The protein isolation methods employed herein may, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor

Laboratory Press, Cold Spring Harbor, New York), which is incorporated herein by reference in its entirety. The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may be a necessary step in the assessment of cells that could be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of a NTP gene.

For example, antibodies, or fragments of antibodies, such as those described, above, in Section 5.3, useful in the present invention may be used to quantitatively or qualitatively detect the presence of NTPs or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody (see below, this Section) coupled with light microscopic, flow cytometric, or fluorimetric detection. Such techniques are especially preferred if such NTP gene products are expressed on the cell surface.

The antibodies (or fragments thereof) or NTP fusion or conjugated proteins useful in the present invention may, additionally, be employed histologically, as in immunofluorescence, immunoelectron microscopy or non-immuno assays, for *in situ* detection of NTPs or conserved variants or peptide fragments thereof, or for NTP binding studies (in the case of labeled fusion proteins incorporating NTP accessory proteins).

*In situ* detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled antibody or fusion protein of the present invention. The antibody (or fragment) or fusion protein is preferably applied by overlaying the labeled antibody (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of a NTP, or conserved variants or peptide fragments, or NTP binding, but also its distribution in the examined tissue. Using the

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conserved variants or peptide fragments thereof, and detecting the bound antibody by any of a number of techniques well-known in the art.

The biological sample may be brought in contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or other solid support which is capable of  
5 immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled NTP antibody or NTP ligand fusion protein. The solid phase support may then be washed with the buffer a second time to remove unbound antibody or fusion protein. The amount of bound label on solid support may then be detected by conventional means.

10 By "solid phase support or carrier" is intended any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material  
15 may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other  
20 suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

The binding activity of a given lot of NTP antibody or NTP ligand fusion protein may be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing  
25 routine experimentation.

With respect to antibodies, one of the ways in which a NTP antibody can be detectably labeled is by linking it to an enzyme that can be used in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD);  
30 Voller, A. *et al.*, 1978, J. Clin. Pathol. 31:507-520; Butler, J.E., 1981, Meth. Enzymol. 73:482-523; Maggio, E. (ed.), 1980, Enzyme Immunoassay, CRC Press, Boca Raton, FL,;

Ishikawa, E. *et al.*, (eds.), 1981, Enzyme Immunoassay, Kigaku Shoin, Tokyo). The enzyme that is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety that can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes  
5 that can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase,  
10 glucoamylase and acetylcholinesterase. The detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

Detection can also be accomplished using any of a variety of other immunoassays.  
15 For example, by radioactively labeling the antibodies or antibody fragments, it is possible to detect NTPs through the use of a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by such means as the use of a gamma  
20 counter or a scintillation counter or by autoradiography.

It is also possible to label the antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin,  
25 allophycocyanin, o-phthaldehyde and fluorescamine.

The antibody can also be detectably labeled using fluorescence emitting metals such as <sup>152</sup>Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

30 The antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by

detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, thermotropic acridinium ester, imidazole, acridinium salt and oxalate ester.

Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

## 10                    5.5        **SCREENING ASSAYS FOR COMPOUNDS THAT MODULATE NTP EXPRESSION OR ACTIVITY**

The following assays are designed to identify compounds that interact with (*e.g.*, bind to) a NTP (including, but not limited to an ECD or CD of a NTP), compounds that interact with (*e.g.*, bind to) intracellular proteins that interact with a NTP (including but not limited to the TM and CD of a NTP), compounds that interfere with the interaction of a NTP with transmembrane or intracellular proteins, or such proteins that may be present in cellular organelles, that are associated with NTP-mediated transport, and to compounds that modulate the activity of a NTP gene (*i.e.*, modulate the level of NTP gene expression) or modulate the quantity of a NTP in the cell. Assays may additionally be utilized that identify compounds that bind to NTP gene regulatory sequences (*e.g.*, promoter sequences) and which may modulate NTP gene expression. See *e.g.*, Platt, K.A., 1994, J. Biol. Chem. 269:28558-28562, which is incorporated herein by reference in its entirety.

The compounds that can be screened in accordance with the invention include but are not limited to peptides, antibodies and fragments thereof, and other organic compounds (*e.g.*, peptidomimetics) that bind to an ECD, or a corresponding nontransmembrane domain of an organelle or nuclear membrane (in the case where the NTP is associated with an intracellular membrane) of the described NTP and either facilitates or inhibits NTP activity; as well as peptides, antibodies or fragments thereof, and other organic compounds that mimic a domain of a NTP (or a portion thereof) and bind to and "neutralize" NTP accessory proteins.

determination can be used to obtain partial or complete geometric structures. The geometric structures may be measured with a complexed ligand, natural or artificial, which may increase the accuracy of the active site structure determined.

If an incomplete or insufficiently accurate structure is determined, the methods of computer based numerical modeling can be used to complete the structure or improve its accuracy. Any recognized modeling method may be used, including parameterized models specific to particular biopolymers such as proteins or nucleic acids, molecular dynamics models based on computing molecular motions, statistical mechanics models based on thermal ensembles, or combined models. For most types of models, standard molecular force fields, representing the forces between constituent atoms and groups, are necessary, and can be selected from force fields known in physical chemistry. The incomplete or less accurate experimental structures can serve as constraints on the complete and more accurate structures computed by these modeling methods.

Finally, having determined the structure of the active site, either experimentally, by modeling, or by a combination, candidate modulating compounds can be identified by searching databases containing compounds along with information on their molecular structure. Such a search seeks compounds having structures that match the determined active site structure and that interact with the groups defining the active site. Such a search can be manual, but is preferably computer assisted. These compounds found from this search are potential NTP modulating compounds.

Alternatively, these methods can be used to identify improved modulating compounds from an already known modulating compound or ligand. The composition of the known compound can be modified and the structural effects of modification can be determined using the experimental and computer modeling methods described above applied to the new composition. The altered structure is then compared to the active site structure of the compound to determine if an improved fit or interaction results. In this manner systematic variations in composition, such as by varying side groups, can be quickly evaluated to obtain modified modulating compounds or ligands of improved specificity or activity.

Further experimental and computer modeling methods useful to identify modulating compounds based upon identification of the active regions of a NTP, and related transport accessory factors will be apparent to those of skill in the art.

Examples of molecular modeling systems are the CHARMM and QUANTA programs (Polygen Corporation, Waltham, MA). CHARMM performs the energy minimization and molecular dynamics functions. QUANTA performs the construction, graphic modeling and analysis of molecular structure. QUANTA allows interactive construction, modification, visualization, and analysis of the behavior of molecules with each other.

10 A number of articles review computer modeling of drugs interactive with specific proteins, such as Rotivinen, *et al.*, 1988, Acta Pharmaceutica Fennica 97:159-166; Ripka, New Scientist 54-57 (June 16, 1988); McKinaly and Rossmann, 1989, Annu. Rev. Pharmacol. Toxicol. 29:111-122; Perry and Davies, OSAR: Quantitative Structure-Activity Relationships in Drug Design pp. 189-193 (Alan R. Liss, Inc. 1989); Lewis and Dean, 1989  
15 Proc. R. Soc. Lond. 236:125-140 and 141-162; and, with respect to a model receptor for nucleic acid components, Askew, *et al.*, 1989, J. Am. Chem. Soc. 111:1082-1090. Other computer programs that screen and graphically depict chemicals are available from companies such as BioDesign, Inc. (Pasadena, CA.), Allelix, Inc. (Mississauga, Ontario, Canada), and Hypercube, Inc. (Cambridge, Ontario). Although these are primarily designed  
20 for application to drugs specific to particular proteins, they can be adapted to design of drugs specific to regions of DNA or RNA, once that region is identified.

Although described above with reference to design and generation of compounds which could alter binding, one could also screen libraries of known compounds, including natural products or synthetic chemicals, and biologically active materials, including proteins,  
25 for compounds which are inhibitors or activators.

Cell-based systems can also be used to identify compounds that bind one of the described NTPs as well as assess the altered activity associated with such binding in living cells. One tool of particular interest for such assays is green fluorescent protein which is described, *inter alia*, in U.S. Patent No. 5,625,048, herein incorporated by reference. Cells  
30 that may be used in such cellular assays include, but are not limited to, leukocytes, or cell lines derived from leukocytes, lymphocytes, stem cells, including embryonic stem cells, and

the like. In addition, expression host cells (e.g., B95 cells, COS cells, CHO cells, OMK cells, fibroblasts, Sf9 cells) genetically engineered to express functional NTP and to respond to activation by the test, or natural, ligand, as measured by a chemical or phenotypic change, or induction of another host cell gene, can be used as an end point in the assay.

5

#### **5.5.1 *IN VITRO* SCREENING ASSAYS FOR COMPOUNDS THAT BIND TO NTPs**

*In vitro* systems may be designed to identify compounds capable of interacting with (e.g., binding to) the described NTPs (including, but not limited to, a ECD or CD of a NTP). Compounds identified may be useful, for example, in modulating the activity of wild type  
10 and/or mutant NTP products; may be useful in elaborating the biological function of a NTP; may be utilized in screens for identifying compounds that disrupt normal NTP functions or interactions; or may in themselves disrupt such interactions.

The principle of the assays used to identify compounds that bind to or interact with a NTP involves preparing a reaction mixture of a NTP and a test compound under conditions  
15 and for a time sufficient to allow the two components to interact and bind, thus forming a complex which can be removed and/or detected in the reaction mixture. The NTP species used can vary depending upon the goal of the screening assay. For such applications, one can use a full length NTP, or a soluble truncated NTP, e.g., in which the TM and/or CD is deleted from the molecule, a peptide corresponding to a ECD or a fusion protein containing  
20 one or more NTP ECD(s) fused to a protein or polypeptide that affords advantages in the assay system (e.g., labeling, isolation of the resulting complex, etc.) can be utilized. Where compounds that interact with the cytoplasmic domain are sought to be identified, peptides corresponding to a NTP CD and fusion proteins containing a NTP CD can be used.

The screening assays can be conducted in a variety of ways. For example, one  
25 method to conduct such an assay would involve anchoring a NTP, NTP polypeptide, peptide or fusion protein, or even the test substance onto a solid phase and detecting NTP/test compound complexes anchored on the solid phase at the end of the reaction. In one embodiment of such a method, the NTP reactant can be anchored onto a solid surface, and the test compound, which is not anchored, may be labeled, either directly or indirectly.

30 In practice, microtiter plates can conveniently be utilized as the solid phase. The anchored component can be immobilized by non-covalent or covalent attachments. Non-

covalent attachment can be accomplished by simply coating the solid surface with a solution of the protein and drying. Alternatively, an immobilized antibody, preferably a monoclonal antibody, specific for the protein to be immobilized may be used to anchor the protein to the solid surface. The surfaces may be prepared in advance and stored.

5 In order to conduct the assay, the nonimmobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously  
10 nonimmobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously nonimmobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the previously nonimmobilized component (the antibody, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig  
15 antibody).

Alternatively, a reaction can be conducted in a liquid phase, the reaction products separated from unreacted components, and complexes detected; *e.g.*, using an immobilized antibody specific for a NTP, NTP polypeptide, peptide or fusion protein or the test compound to anchor any complexes formed in solution, and a labeled antibody specific for  
20 the other component of the possible complex to detect anchored complexes.

Alternatively, cell-based assays can be used to identify compounds that interact with NTPs. To this end, cell lines that express a NTP, or cell lines (*e.g.*, COS cells, CHO cells, fibroblasts, etc.) that have been genetically engineered to express a NTP (*e.g.*, by transfection or transduction of NTP gene DNA) can be used. Interaction of the test  
25 compound with, for example, an ECD from a NTP expressed by the host cell can be determined by comparison or competition with native ligand.

### 5.5.2 ASSAYS FOR INTRACELLULAR PROTEINS THAT INTERACT WITH NTPs

Any method suitable for detecting protein-protein interactions may be employed for  
30 identifying transmembrane proteins or intracellular proteins that interact with the described

NTPs. Among the traditional methods which can be employed are co-immunoprecipitation, crosslinking and co-purification through gradients or chromatographic columns of cell lysates or proteins obtained from cell lysates to identify proteins in the lysate that interact with a NTP. For these assays, the NTP component used can be a full length NTP, a soluble  
5 derivative lacking the membrane-anchoring region (*e.g.*, a truncated NTP in which a TM is deleted resulting in a truncated molecule containing a ECD fused to a CD), a peptide corresponding to a CD or a fusion protein containing a CD from the described NTP. Once isolated, such an intracellular protein can be identified and can, in turn, be used, in conjunction with standard techniques, to identify proteins with which it interacts. For  
10 example, at least a portion of the amino acid sequence of an intracellular protein that interacts with a NTP can be ascertained using techniques well known to those of skill in the art, such as via the Edman degradation technique. (See, *e.g.*, Creighton, 1983, "Proteins: Structures and Molecular Principles", W.H. Freeman & Co., N.Y., pp.34-49). The amino acid sequence obtained can be used as a guide for the generation of oligonucleotide mixtures  
15 that can be used to screen for gene sequences encoding such intracellular proteins. Screening may be accomplished, for example, by standard hybridization or PCR techniques. Techniques for the generation of oligonucleotide mixtures and the screening are well-known. (See, *e.g.*, Ausubel, *supra*, and PCR Protocols: A Guide to Methods and Applications, 1990, Innis, M. *et al.*, eds. Academic Press, Inc., New York).

20 Additionally, methods may be employed which result in the simultaneous identification of genes which encode the transmembrane or intracellular proteins interacting with a NTP. These methods include, for example, probing expression, libraries, in a manner similar to the well known technique of antibody probing of  $\lambda$ gt11 libraries, using labeled NTPs, or a NTP polypeptide, peptide or fusion protein, *e.g.*, a NTP polypeptide or a NTP  
25 domain fused to a marker (*e.g.*, an enzyme, fluor, luminescent protein, or dye), or an Ig-Fc domain.

One method that detects protein interactions *in vivo*, the two-hybrid system, is described in detail for illustration only and not by way of limitation. One version of this system has been described (Chien *et al.*, 1991, Proc. Natl. Acad. Sci. USA, 88:9578-9582)  
30 and is commercially available from Clontech (Palo Alto, CA).

Briefly, utilizing such a system, plasmids are constructed that encode two hybrid proteins: one plasmid consists of nucleotides encoding the DNA-binding domain of a transcription activator protein fused to nucleotide sequence encoding a NTP, or a NTP polypeptide, peptide or fusion protein, and the other plasmid includes nucleotides encoding the transcription activator protein's activation domain fused to a cDNA encoding an unknown protein which has been recombined into this plasmid as part of a cDNA library. The DNA-binding domain fusion plasmid and the cDNA library are transformed into a strain of the yeast *Saccharomyces cerevisiae* that contains a reporter gene (e.g., HBS or *lacZ*) whose regulatory region contains the transcription activator's binding site. Either hybrid protein alone cannot activate transcription of the reporter gene: the DNA-binding domain hybrid cannot because it does not provide activation function and the activation domain hybrid cannot because it cannot localize to the activator's binding sites. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product.

The two-hybrid system or related methodology can be used to screen activation domain libraries for proteins that interact with the "bait" gene product. By way of example, and not by way of limitation, a NTP may be used as the bait gene product. Total genomic or cDNA sequences are fused to the DNA encoding an activation domain. This library and a plasmid encoding a hybrid of the bait NTP product fused to the DNA-binding domain are cotransformed into a yeast reporter strain, and the resulting transformants are screened for those that express the reporter gene. For example, and not by way of limitation, a bait NTP gene sequence, such as the open reading frame of the NTP (or a domain of the NTP) can be cloned into a vector such that it is translationally fused to the DNA encoding the DNA-binding domain of the GAL4 protein. These colonies are purified and the library plasmids responsible for reporter gene expression are isolated. DNA sequencing is then used to identify the proteins encoded by the library plasmids.

A cDNA library of the cell line from which proteins that interact with bait NTP product are to be detected can be made using methods routinely practiced in the art. According to the particular system described herein, for example, the cDNA fragments can be inserted into a vector such that they are translationally fused to the transcriptional activation domain of GAL4. This library can be co-transformed along with the bait NTP

gene-GAL4 fusion plasmid into a yeast strain which contains a lacZ gene driven by a promoter which contains GAL4 activation sequence. A cDNA encoded protein, fused to GAL4 transcriptional activation domain, that interacts with a bait NTP product will reconstitute an active GAL4 protein and thereby drive expression of the HIS3 gene.

- 5 Colonies that express HIS3 can be detected by their growth on petri dishes containing semi-solid agar based media lacking histidine. The cDNA can then be purified from these strains, and used to produce and isolate the bait NTP gene-interacting protein using techniques routinely practiced in the art.

### 10 5.5.3 ASSAYS FOR COMPOUNDS THAT INTERFERE WITH NTP/INTRACELLULAR OR NTP/MEMBRANE MACROMOLECULE INTERACTIONS

- The macromolecules that interact with a NTP are referred to, for purposes of this discussion, as "binding partners". These binding partners are likely to be involved in NTP-mediated transport. Therefore, it is desirable to identify compounds that interfere with or  
15 disrupt the interaction of such binding partners that can be useful in regulating the activity of NTP and controlling disorders associated with NTP activity.

- The basic principle of the assay systems used to identify compounds that interfere with the interaction between NTP and any binding partner or partners involves preparing a reaction mixture containing a NTP, NTP polypeptide, peptide or fusion protein as described  
20 in Sections 5.5.1 and 5.5.2 above, and the binding partner under conditions and for a time sufficient to allow the two to interact and bind, thus forming a complex. In order to test a compound for inhibitory activity, the reaction mixture is prepared in the presence and absence of the test compound. The test compound may be initially included in the reaction mixture, or may be added at a time subsequent to the addition of the NTP moiety and its  
25 binding partner. Control reaction mixtures are incubated without the test compound or with a placebo. The formation of any complexes between the NTP moiety and the binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction of the NTP and the interactive binding partner. Additionally, complex  
30 formation within reaction mixtures containing the test compound and normal NTP may also be compared to complex formation within reaction mixtures containing the test compound

and a mutant NTP. This comparison may be important in those cases wherein it is desirable to identify compounds that specifically disrupt interactions of mutant, or mutated, NTPs but not normal NTPs.

The assay for compounds that interfere with the interaction of the described NTP and binding partners can be conducted in a heterogeneous or homogeneous format.

Heterogeneous assays involve anchoring either the NTP moiety product or the binding partner onto a solid phase and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different

information about the compounds being tested. For example, test compounds that interfere with the interaction by competition can be identified by conducting the reaction in the presence of the test substance; *i.e.*, by adding the test substance to the reaction mixture prior to, or simultaneously with, the NTP moiety and interactive binding partner. Alternatively, test compounds that disrupt preformed complexes, *e.g.* compounds with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are described briefly below.

In a heterogeneous assay system, either the NTP moiety or an interactive binding partner, is anchored onto a solid surface, while the non-anchored species is labeled, either directly or indirectly. In practice, microtiter plates are conveniently utilized. The anchored species may be immobilized by non-covalent or covalent attachments. Non-covalent attachment may be accomplished simply by coating the solid surface with a solution of NTP product or binding partner and drying. Alternatively, an immobilized antibody specific for the species to be anchored may be used to anchor the species to the solid surface. The surfaces may be prepared in advance and stored.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) and any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were

formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody). Depending upon the order of addition of reaction  
5 components, test compounds which inhibit complex formation or which disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; *e.g.*, using an immobilized antibody specific for one of the binding  
10 components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds which inhibit complex or which disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. In  
15 this approach, a preformed complex of a NTP moiety and an interactive binding partner is prepared in which either the NTP or its binding partners is labeled, but the signal generated by the label is quenched due to formation of the complex (see, *e.g.*, U.S. Patent No. 4,109,496 by Rubenstein which utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed  
20 complex will result in the generation of a signal above background. In this way, test substances which disrupt NTP/binding partner interaction can be identified.

In a particular embodiment, a NTP fusion can be prepared for immobilization. For example, a NTP or a peptide fragment, *e.g.*, corresponding to a CD, can be fused to a glutathione-S-transferase (GST) gene using a fusion vector, such as pGEX-5X-1, in such a  
25 manner that its binding activity is maintained in the resulting fusion protein. The interactive binding partner can be purified and used to raise a monoclonal antibody using methods routinely practiced in the art and described above, in Section 5.3. This antibody can be labeled with the radioactive isotope <sup>125</sup>I, for example, by methods routinely practiced in the art. In a heterogeneous assay, *e.g.*, the GST-NTP fusion protein can be anchored to  
30 glutathione-agarose beads. The interactive binding partner can then be added in the presence or absence of the test compound in a manner that allows interaction and binding to

occur. At the end of the reaction period, unbound material can be washed away, and the labeled monoclonal antibody can be added to the system and allowed to bind to the complexed components. The interaction between the NTP product and the interactive binding partner can be detected by measuring the amount of radioactivity that remains  
5 associated with the glutathione-agarose beads. A successful inhibition of the interaction by the test compound will result in a decrease in measured radioactivity.

Alternatively, the GST-NTP fusion protein and the interactive binding partner can be mixed together in liquid in the absence of the solid glutathione-agarose beads. The test compound can be added either during or after the species are allowed to interact. This  
10 mixture can then be added to the glutathione-agarose beads and unbound material is washed away. Again the extent of inhibition of the NTP/binding partner interaction can be detected by adding the labeled antibody and measuring the radioactivity associated with the beads.

In another embodiment of the invention, these same techniques can be employed using peptide fragments that correspond to the binding domains of a NTP and/or the  
15 interactive or binding partner (in cases where the binding partner is a protein), in place of one or both of the full length proteins. Any number of methods routinely practiced in the art can be used to identify and isolate the binding sites. These methods include, but are not limited to, mutagenesis of the gene encoding one of the proteins and screening for disruption of binding in a co-immunoprecipitation assay. Compensatory mutations in the gene  
20 encoding the second species in the complex can then be selected. Sequence analysis of the genes encoding the respective proteins will reveal the mutations that correspond to the region of the protein involved in interactive binding. Alternatively, one protein can be anchored to a solid surface using methods described above, and allowed to interact with and bind to its labeled binding partner, which has been treated with a proteolytic enzyme, such  
25 as trypsin. After washing, a relatively short, labeled peptide comprising the binding domain may remain associated with the solid material, which can be isolated and identified by amino acid sequencing. Also, once the gene coding for the intracellular binding partner is obtained, short gene segments can be engineered to express peptide fragments of the protein, which can then be tested for binding activity and purified or synthesized.

30 For example, and not by way of limitation, a NTP product can be anchored to a solid material as described, above, by making a GST-NTP fusion protein and allowing it to bind

to glutathione agarose beads. The interactive binding partner can be labeled with a radioactive isotope, such as  $^{35}\text{S}$ , and cleaved with a proteolytic enzyme such as trypsin. Cleavage products can then be added to the anchored GST-NTP fusion protein and allowed to bind. After washing away unbound peptides, labeled bound material, representing the  
5 intracellular binding partner binding domain, can be eluted, purified, and analyzed for amino acid sequence by well-known methods. Peptides so identified can be produced synthetically or fused to appropriate facilitative proteins using recombinant DNA technology.

## 5.6 MODULATORY, ANTISENSE, RIBOZYME AND TRIPLE HELIX APPROACHES

10 In another embodiment, the levels of NTP gene regulation can be reduced by using well-known antisense, gene "knock-out," ribozyme and/or triple helix methods. Such molecules may be designed to modulate, reduce or inhibit either unimpaired, or if appropriate, mutant sequence activity. Techniques for the production and use of such molecules are well known to those of skill in the art.

15 Antisense RNA and DNA molecules act to directly block the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. Antisense approaches involve the design of oligonucleotides which are complementary to an mRNA sequence. The antisense oligonucleotides will bind to the complementary mRNA sequence transcripts and prevent translation. Absolute complementarity, although preferred, is not required.

20 A sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense  
25 nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

In one embodiment, oligonucleotides complementary to non-coding regions of the  
30 sequence of interest could be used in an antisense approach to inhibit translation of endogenous mRNA. Antisense nucleic acids should be at least six nucleotides in length, and

are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

Regardless of the choice of target sequence, it is preferred that *in vitro* studies are first performed to quantitate the ability of the antisense oligonucleotide to inhibit mRNA expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide and that the nucleic acid of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger, *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre, *et al.*, 1987, *Proc. Natl. Acad. Sci. U.S.A.* 84:648-652; PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, *e.g.*, PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents (see, *e.g.*, Krol *et al.*, 1988, *BioTechniques* 6:958-976) or intercalating agents (see, *e.g.*, Zon, 1988, *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine,

2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine,  
 5 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose,  
 10 and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

15 In yet another embodiment, the antisense oligonucleotide is an  $\alpha$ -anomeric oligonucleotide. An  $\alpha$ -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other (Gautier, *et al.*, 1987, *Nucl. Acids Res.* 15:6625-6641). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue, *et al.*, 1987, *Nucl. Acids Res.* 15:6131-6148), or a  
 20 chimeric RNA-DNA analogue (Inoue, *et al.*, 1987, *FEBS Lett.* 215:327-330).

Oligonucleotides of the invention may be synthesized by standard methods known in the art, *e.g.*, by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein, *et al.* (1988, *Nucl. Acids Res.*  
 25 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin, *et al.*, 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85:7448-7451), *etc.*

While antisense nucleotides complementary to a coding region sequence could be used, those complementary to the transcribed, untranslated region are most preferred.

Antisense molecules should be delivered to cells that express the sequence *in vivo*.  
 30 A number of methods have been developed for delivering antisense DNA or RNA to cells; *e.g.*, antisense molecules can be injected directly into the tissue site, or modified antisense

molecules, designed to target the desired cells (*e.g.*, antisense linked to peptides or antibodies which specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

A preferred approach to achieve intracellular concentrations of the antisense  
5 sufficient to suppress translation of endogenous mRNAs utilizes a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong pol III or pol II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs which will form complementary base pairs with the endogenous sequence transcripts and thereby prevent  
10 translation of the mRNA sequence. For example, a vector can be introduced *e.g.*, such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in  
15 the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3'-long terminal repeat of Rous  
20 sarcoma virus (Yamamoto, *et al.*, 1980, *Cell* 22:787-797), the herpes thymidine kinase promoter (Wagner, *et al.*, 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster, *et al.*, 1982, *Nature* 296:39-42), etc. Any type of plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct that can be introduced directly into the tissue site. Alternatively, viral vectors can  
25 be used that selectively infect the desired tissue, in which case administration may be accomplished by another route (*e.g.*, systemically).

Ribozyme molecules designed to catalytically cleave target gene mRNA transcripts can also be used to prevent translation of target gene mRNA and, therefore, expression of target gene product. (See, *e.g.*, PCT International Publication WO90/11364, published  
30 October 4, 1990; Sarver, *et al.*, 1990, *Science* 247, 1222-1225).

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. (For a review, see Rossi, 1994, *Current Biology* 4:469-471). The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage event.

- 5 The composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA, and must include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see, *e.g.*, U.S. Patent No. 5,093,246, which is incorporated herein by reference in its entirety.

- While ribozymes that cleave mRNA at site specific recognition sequences can be  
10 used to destroy target gene mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions which form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in  
15 Myers, 1995, *Molecular Biology and Biotechnology: A Comprehensive Desk Reference*, VCH Publishers, New York, (see especially Figure 4, page 833) and in Haseloff and Gerlach, 1988, *Nature*, 334:585-591, which is incorporated herein by reference in its entirety.

- Preferably the ribozyme is engineered so that the cleavage recognition site is located  
20 near the 5' end of the target gene mRNA, *i.e.*, to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

- The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one which occurs naturally in *Tetrahymena thermophila* (known as the IVS, or L-19 IVS RNA) and which has been extensively  
25 described by Thomas Cech and collaborators (Zaug, *et al.*, 1984, *Science*, 224:574-578; Zaug and Cech, 1986, *Science*, 231:470-475; Zaug, *et al.*, 1986, *Nature*, 324:429-433; published International patent application No. WO 88/04300 by University Patents Inc.; Been and Cech, 1986, *Cell*, 47:207-216). The Cech-type ribozymes have an eight base pair active site that hybridizes to a target RNA sequence whereafter cleavage of the target RNA  
30 takes place. The invention encompasses those Cech-type ribozymes that target eight base-pair active site sequences that are present in the target gene.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (*e.g.*, for improved stability, targeting, *etc.*) and should be delivered to cells that express the target gene *in vivo*. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous target gene messages and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Endogenous target gene expression can also be reduced by inactivating or "knocking out" the target gene or its promoter using targeted homologous recombination (*e.g.*, see Smithies, *et al.*, 1985, *Nature* 317:230-234; Thomas and Capecchi, 1987, *Cell* 51:503-512; Thompson, *et al.*, 1989, *Cell* 5:313-321; each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional target gene (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous target gene (either the coding regions or regulatory regions of the target gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells which express the target gene *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the target gene. Such approaches are particularly suited in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive target gene (*e.g.*, see Thomas and Capecchi, 1987 and Thompson, 1989, *supra*). However this approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors.

Alternatively, endogenous target gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the target gene (*i.e.*, the target gene promoter and/or enhancers) to form triple helical structures which prevent transcription of the target gene in target cells in the body. (See generally, Helene, 1991, *Anticancer Drug Des.*, 6(6):569-584; Helene, *et al.*, 1992, *Ann. N.Y. Acad. Sci.*, 660:27-36; and Maher, 1992, *Bioassays* 14(12):807-815).

Nucleic acid molecules to be used in triple helix formation for the inhibition of transcription should be single stranded and composed of deoxynucleotides. The base

composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, which generally require sizable stretches of either purines or pyrimidines to be present on one strand of a duplex. Nucleic acids may be pyrimidine-based, which will result in TAT and CGC<sup>+</sup> triplets across the three associated strands of the  
5 resulting triple helix. The pyrimidine-rich molecules provide base complementarity to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, nucleic acid molecules may be chosen which are purine-rich, for example, contain a stretch of G residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand  
10 of the targeted duplex, resulting in GGC triplets across the three strands in the triplex.

Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so-called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizable stretch  
15 of either purines or pyrimidines to be present on one strand of a duplex.

In instances wherein the antisense, ribozyme, and/or triple helix molecules described herein are utilized to inhibit mutant gene expression, it is possible that the technique may so efficiently reduce or inhibit the transcription (triple helix) and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles which the possibility may arise  
20 wherein the concentration of normal target gene product present may be lower than is necessary for a normal phenotype. Alternatively, in instances whereby the target gene encodes an extracellular protein, it may be preferable to co-administer normal target gene protein in order to maintain the requisite level of target gene activity.

Anti-sense RNA and DNA, ribozyme, and triple helix molecules of the invention  
25 may be prepared by any method known in the art for the synthesis of DNA and RNA molecules, as discussed above. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as for example solid-phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the  
30 antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6

polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

## 5.7 PHARMACEUTICAL FORMULATIONS AND METHODS OF ADMINISTRATION

The compounds of this invention can be formulated and administered to inhibit a variety of disease states by any means that produces contact of the active ingredient with the agent's site of action in the body of a mammal. They can be administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic active ingredients or in a combination of therapeutic active ingredients. They can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

The dosage administered will be a therapeutically effective amount of the compound sufficient to result in amelioration of symptoms of the disease and will, of course, vary depending upon known factors such as the pharmacodynamic characteristics of the particular active ingredient and its mode and route of administration; age, sex, health and weight of the recipient; nature and extent of symptoms; kind of concurrent treatment, frequency of treatment and the effect desired.

Preferably, agents that modulate NTP function shall be substantially specific. For the purposes of the present invention, the term substantially specific shall mean that a given agent is capable of being dosaged to provide the desired effect while not causing undue cellular toxicity.

One of ordinary skill will appreciate that, from a medical practitioner's or patient's perspective, virtually any alleviation or prevention of an undesirable symptom (e.g., symptoms related to disease, sensitivity to environmental factors, normal aging, and the like) would be desirable. Thus, for the purposes of this Application, the terms "treatment", "therapeutic use", or "medicinal use" used herein shall refer to any and all uses of compositions comprising the claimed agents which remedy a disease state or symptoms, or otherwise prevent, hinder, retard, or reverse the progression of disease or other undesirable symptoms in any way whatsoever.

When used in the therapeutic treatment of disease, an appropriate dosage of presently described agents, or derivatives thereof, may be determined by any of several well established methodologies. For instance, animal studies are commonly used to determine the maximal tolerable dose, or MTD, of bioactive agent per kilogram weight. In general, at least one of the animal species tested is mammalian. Those skilled in the art regularly extrapolate doses for efficacy and avoiding toxicity to other species, including human. Before human studies of efficacy are undertaken, Phase I clinical studies in normal subjects help establish safe doses.

Additionally, the bioactive agents may be complexed with a variety of well established compounds or structures that, for instance, enhance the stability of the bioactive agent, or otherwise enhance its pharmacological properties (e.g., increase *in vivo* half-life, reduce toxicity, etc.).

#### 5.7.1 DOSE DETERMINATIONS

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the  $LD_{50}$  (the dose lethal to 50% of the population) and the  $ED_{50}$  (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio  $LD_{50}/ED_{50}$ . Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the  $ED_{50}$  with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the  $IC_{50}$  (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such

information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

Specific dosages may also be utilized for antibodies. Typically, the preferred dosage is 0.1 mg/kg to 100 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg), and if the  
5 antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate. If the antibody is partially human or fully human, it generally will have a longer half-life within the human body than other antibodies. Accordingly, lower dosages of partially human and fully human antibodies is often possible. Additional modifications may be used to further stabilize antibodies. For example, lipidation can be used to stabilize antibodies  
10 and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of antibodies is described by Cruikshank *et al.* ((1997) *J. Acquired Immune Deficiency Syndromes and Human Retrovirology* 14:193).

A therapeutically effective amount of protein or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25  
15 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight.

Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide or antibody can include a single treatment or, preferably, can include a series of  
20 treatments. In a preferred example, a subject is treated with antibody, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5 or 6 weeks.

The present invention further encompasses agents which modulate expression or  
25 activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e.*, including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or  
30 inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per

mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

It is understood that appropriate doses of small molecule agents depends upon a number of factors known to those of ordinary skill in the art, *e.g.*, a physician. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention. Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram).

### 5.7.2 FORMULATIONS AND USE

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

Thus, the compounds and their physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as

suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, 5 flavoring, coloring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

10 For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined 15 by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.* gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in 20 unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use. In general, 25 water, a suitable oil, saline, aqueous dextrose (glucose), and related sugar solutions and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Solutions for parenteral administration contain preferably a water soluble salt of the active ingredient, suitable stabilizing agents and, if necessary, buffer substances. Antioxidizing agents such as sodium bisulfate, sodium sulfite or ascorbic acid, either alone 30 or combined, are suitable stabilizing agents. Also used are citric acid and its salts and sodium ethylenediaminetetraacetic acid (EDTA). In addition, parenteral solutions can

contain preservatives such as benzalkonium chloride, methyl- or propyl-paraben and chlorobutanol. Suitable pharmaceutical carriers are described in *Remington's Pharmaceutical Sciences*, a standard reference text in this field.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Additionally, standard pharmaceutical methods can be employed to control the duration of action. These are well known in the art and include control release preparations and can include appropriate macromolecules, for example polymers, polyesters, polyamino acids, polyvinyl, pyrrolidone, ethylenevinylacetate, methyl cellulose, carboxymethyl cellulose or protamine sulfate. The concentration of macromolecules as well as the methods of incorporation can be adjusted in order to control release. Additionally, the agent can be incorporated into particles of polymeric materials such as polyesters, polyamino acids, hydrogels, poly (lactic acid) or ethylenevinylacetate copolymers. In addition to being incorporated, these agents can also be used to trap the compound in microcapsules.

Another aspect of the present invention includes formulations that provide for the sustained release of NTP antagonists. Examples of such sustained release formulations include composites of biocompatible polymers, such as poly(lactic acid), poly(lactic-co-glycolic acid), methylcellulose, hyaluronic acid, collagen, and the like. The structure, selection and use of degradable polymers in drug delivery vehicles have been reviewed in several publications, including, A. Domb *et al.*, Polymers for Advanced Technologies 3:279-292 (1992). Additional guidance in selecting and using polymers in pharmaceutical formulations can be found in the text by M. Chasin and R. Langer (eds.), "Biodegradable Polymers as Drug Delivery Systems," Vol. 45 of "Drugs and the Pharmaceutical Sciences," M. Dekker, New York, 1990. Liposomes may also be used to provide for the sustained release of NTP antagonists. Details concerning how to use and make liposomal

formulations of drugs of interest can be found in, among other places, U.S. Pat. No 4,944,948; U.S. Pat. No. 5,008,050; U.S. Pat. No. 4,921,706; U.S. Pat. No. 4,927,637; U.S. Pat. No. 4,452,747; U.S. Pat. No. 4,016,100; U.S. Pat. No. 4,311,712; U.S. Pat. No. 4,370,349; U.S. Pat. No. 4,372,949; U.S. Pat. No. 4,529,561; U.S. Pat. No. 5,009,956; U.S. Pat. No. 4,725,442; U.S. Pat. No. 4,737,323; U.S. Pat. No. 4,920,016. Sustained release formulations are of particular interest when it is desirable to provide a high local concentration of NTP antagonist.

Where diagnostic, therapeutic or medicinal use of the presently described agents, or derivatives thereof, is contemplated, the bioactive agents may be introduced *in vivo* by any of a number of established methods. For instance, the agent may be administered by inhalation; by subcutaneous (sub-q); intravenous (I.V.), intraperitoneal (I.P.), or intramuscular (I.M.) injection; or as a topically applied agent (transdermal patch, ointments, creams, salves, eye drops, and the like).

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

Useful pharmaceutical dosage forms, for administration of the compounds of this invention can be illustrated as follows:

20        Capsules: Capsules are prepared by filling standard two-piece hard gelatin capsules each with the desired amount of powdered active ingredient, 175 milligrams of lactose, 24 milligrams of talc and 6 milligrams magnesium stearate.

Soft Gelatin Capsules: A mixture of active ingredient in soybean oil is prepared and injected by means of a positive displacement pump into gelatin to form soft gelatin capsules containing the desired amount of the active ingredient. The capsules are then washed and dried.

Tablets: Tablets are prepared by conventional procedures so that the dosage unit is the desired amount of active ingredient. 0.2 milligrams of colloidal silicon dioxide, 5 milligrams of magnesium stearate, 275 milligrams of microcrystalline cellulose, 11 milligrams of cornstarch and 98.8 milligrams of lactose. Appropriate coatings may be applied to increase palatability or to delay absorption.

Injectable: A parenteral composition suitable for administration by injection is prepared by stirring 1.5% by weight of active ingredients in 10% by volume propylene glycol and water. The solution is made isotonic with sodium chloride and sterilized.

Suspension: An aqueous suspension is prepared for oral administration so that each 5 millimeters contain 100 milligrams of finely divided active ingredient, 200 milligrams of sodium carboxymethyl cellulose, 5 milligrams of sodium benzoate, 1.0 grams of sorbitol solution U.S.P. and 0.025 millimeters of vanillin.

Accordingly, the pharmaceutical composition of the present invention may be delivered via various routes and to various sites in an animal body to achieve a particular effect (see, *e.g.*, Rosenfeld *et al.* (1991), *supra*; Rosenfeld *et al.*, Clin. Res., 39(2), 311A (1991 a); Jaffe *et al.*, *supra*; Berkner, *supra*). One skilled in the art will recognize that although more than one route can be used for administration, a particular route can provide a more immediate and more effective reaction than another route. Local or systemic delivery can be accomplished by administration comprising application or instillation of the formulation into body cavities, inhalation or insufflation of an aerosol, or by parenteral introduction, comprising intramuscular, intravenous, peritoneal, subcutaneous, intradermal, as well as topical administration.

The composition of the present invention can be provided in unit dosage form wherein each dosage unit, *e.g.*, a teaspoonful, tablet, solution, or suppository, contains a predetermined amount of the composition, alone or in appropriate combination with other active agents. The term "unit dosage form" as used herein refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of the compositions of the present invention, alone or in combination with other active agents, calculated in an amount sufficient to produce the desired effect, in association with a pharmaceutically acceptable diluent, carrier, or vehicle, where appropriate. The specifications for the unit dosage forms of the present invention depend on the particular effect to be achieved and the particular pharmacodynamics associated with the pharmaceutical composition in the particular host.

These methods described herein are by no means all-inclusive, and further methods to suit the specific application will be apparent to the ordinary skilled artisan. Moreover, the

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes a novel transporter protein having the amino acid sequence of SEQ ID NO:2, SEQ  
5 ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30 or SEQ ID NO:32.
2. The isolated nucleic acid molecule of Claim 1 comprising the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9,  
10 SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29 or SEQ ID NO:31.
3. An isolated nucleic acid molecule comprising a complement of the nucleic acid molecule of Claim 1.
4. An isolated nucleic acid molecule comprising at least 24 contiguous bases of  
15 the nucleic acid molecule of Claim 2, or the complement thereof.
5. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes at least fifty contiguous amino acids of the amino acid sequence shown in SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID  
20 NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30 or SEQ ID NO:32.
6. An isolated nucleic acid molecule which hybridizes to the nucleic acid molecule as in one of claims 1-5 under highly stringent conditions.
7. An isolated nucleic acid molecule which hybridizes to the nucleic acid molecule as in one of claims 1-5 under moderately stringent conditions.

8. A vector comprising the nucleic acid molecule of Claim 6.

9. An expression vector comprising the nucleic acid molecule of Claim 6 operatively associated with a regulatory nucleic acid controlling the expression of the nucleic acid in a host cell.

5 10. A host cell genetically engineered to express the nucleic acid molecule of Claim 6.

11. A host cell genetically engineered to express the nucleic acid molecule of Claim 6 operatively associated with a regulatory nucleic acid controlling the expression of the nucleic acid in the host cell.

10 12. A transgenic, non-human animal, which has been genetically engineered to contain a transgene comprising the nucleic acid molecule of Claim 6.

13. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID  
15 NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30 or SEQ ID NO:32.

14. An isolated polypeptide comprising an amino acid sequence encoded by the isolated nucleic acid molecule of Claim 6.

15. An isolated polypeptide comprising an amino acid sequence encoded by the isolated nucleic acid molecule of Claim 2.

20 16. An antibody which binds to an isolated polypeptide comprising an amino acid sequence encoded by the isolated nucleic acid molecule of Claim 6.

17. A method for identifying a compound which modulates expression of a novel transporter protein comprising (NTP):

- (a) contacting a test compound to a cell that expresses an NTP;
- (b) measuring a level of the NTP expression in the cell; and
- 5 (c) comparing the level of the NTP expression in the cell in the presence of the test compound to a level of the NTP expression in the cell in the absence of the test compound;

wherein, if the level of the NTP expression in the cell in the presence of the test compound differs from the level of expression of the NTP in the cell in the absence of the test  
10 compound, a compound that modulates expression of the NTP is identified.

18. A method for transferring a novel transporter protein (NTP) in a cell comprising contacting the cell with a nucleic acid comprising an NTP such that the NTP is transferred into the cell.

19. The method of Claim 17 wherein the NTP is expressed in the cell.

## SEQUENCE LISTING

<110> Turner, Alex  
Zambrowicz, Brian  
Nehls, Michael  
Friedrich, Glenn  
Sands, Arthur T.

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&lt;211&gt; 627

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

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gcctaa						1746

&lt;210&gt; 6

&lt;211&gt; 581

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 6

Met	Gly	Leu	Leu	Gln	Gly	Leu	Leu	Arg	Val	Arg	Lys	Leu	Leu	Leu	Val
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Val	Cys	Val	Pro	Leu	Leu	Leu	Leu	Pro	Leu	Pro	Val	Leu	His	Pro	Ser
			20					25					30		
Ser	Glu	Ala	Ser	Cys	Ala	Tyr	Val	Leu	Ile	Val	Thr	Ala	Val	Tyr	Trp
		35					40					45			
Val	Ser	Glu	Ala	Val	Pro	Leu	Gly	Ala	Ala	Ala	Leu	Val	Pro	Ala	Phe
		50				55					60				
Leu	Tyr	Pro	Phe	Phe	Gly	Val	Leu	Arg	Ser	Asn	Glu	Val	Ala	Ala	Glu
65					70				75				80		
Tyr	Phe	Lys	Asn	Thr	Thr	Leu	Leu	Leu	Val	Gly	Val	Ile	Cys	Val	Ala
			85					90					95		
Ala	Ala	Val	Glu	Lys	Trp	Asn	Leu	His	Lys	Arg	Ile	Ala	Leu	Arg	Met
		100					105					110			
Val	Leu	Met	Ala	Gly	Ala	Lys	Pro	Gly	Met	Leu	Leu	Leu	Cys	Phe	Met
		115					120					125			
Cys	Cys	Thr	Thr	Leu	Leu	Ser	Met	Trp	Leu	Ser	Asn	Thr	Ser	Thr	Thr

	130					135					140						
Ala	Met	Val	Met	Pro	Ile	Val	Glu	Ala	Val	Leu	Gln	Glu	Leu	Val	Ser		
145						150					155					160	
Ala	Glu	Asp	Glu	Gln	Leu	Val	Ala	Gly	Asn	Ser	Asn	Thr	Glu	Glu	Ala		
						165					170					175	
Glu	Pro	Ile	Ser	Leu	Asp	Val	Lys	Asn	Ser	Gln	Pro	Ser	Leu	Glu	Leu		
						180					185					190	
Ile	Phe	Val	Asn	Glu	Asp	Arg	Ser	Asn	Ala	Asp	Leu	Thr	Thr	Leu	Met		
						195					200					205	
His	Asn	Glu	Asn	Leu	Asn	Gly	Val	Pro	Ser	Ile	Thr	Asn	Pro	Ile	Lys		
						210					215					220	
Thr	Ala	Asn	Gln	His	Gln	Gly	Lys	Lys	Gln	His	Pro	Ser	Gln	Glu	Lys		
225						230					235					240	
Pro	Gln	Val	Leu	Thr	Pro	Ser	Pro	Arg	Lys	Gln	Lys	Leu	Asn	Arg	Lys		
						245					250					255	
Tyr	Arg	Ser	His	His	Asp	Gln	Met	Ile	Cys	Lys	Cys	Leu	Ser	Leu	Ser		
						260					265					270	
Ile	Ser	Tyr	Ser	Ala	Thr	Ile	Gly	Gly	Leu	Thr	Thr	Ile	Ile	Gly	Thr		
						275					280					285	
Ser	Thr	Ser	Leu	Ile	Phe	Leu	Glu	His	Phe	Asn	Asn	Gln	Tyr	Pro	Ala		
						290					295					300	
Ala	Glu	Val	Val	Asn	Phe	Gly	Thr	Trp	Phe	Leu	Phe	Ser	Phe	Pro	Ile		
305						310					315					320	
Ser	Leu	Ile	Met	Leu	Val	Val	Ser	Trp	Phe	Trp	Met	His	Trp	Leu	Phe		
						325					330					335	
Leu	Gly	Cys	Asn	Phe	Lys	Glu	Thr	Cys	Ser	Leu	Ser	Lys	Lys	Lys	Lys		
						340					345					350	
Thr	Lys	Arg	Glu	Gln	Leu	Ser	Glu	Lys	Arg	Ile	Gln	Glu	Glu	Tyr	Glu		
						355					360					365	
Lys	Leu	Gly	Asp	Ile	Ser	Tyr	Pro	Glu	Met	Val	Thr	Gly	Phe	Phe	Phe		
						370					375					380	
Ile	Leu	Met	Thr	Val	Leu	Trp	Phe	Thr	Arg	Glu	Pro	Gly	Phe	Val	Pro		
385						390					395					400	
Gly	Trp	Asp	Ser	Phe	Phe	Glu	Lys	Lys	Gly	Tyr	Arg	Thr	Asp	Ala	Thr		
						405					410					415	
Val	Ser	Val	Phe	Leu	Gly	Phe	Leu	Leu	Phe	Leu	Ile	Pro	Ala	Lys	Lys		
						420					425					430	
Pro	Cys	Phe	Gly	Lys	Lys	Asn	Asp	Gly	Glu	Asn	Gln	Glu	His	Ser	Leu		
						435					440					445	
Gly	Thr	Glu	Pro	Ile	Ile	Thr	Trp	Lys	Asp	Phe	Gln	Lys	Thr	Met	Pro		
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Ile Thr Asp Gln Ala  
580

<210> 7  
<211> 1884  
<212> DNA  
<213> Homo sapiens

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ctgatcgtga ctgctgtgta ctgggtgtcg gaggcagtgc ctctgggagc tgcagccctg 180  
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aagtggaaacc tgcataagcg cattgctctg cgcattggtt tgatggccgg rgccaagccg 360  
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aaccatca aaactgcaaa ccaacaccag ggcaagaagc aacacccatc ccaggaaaag 720  
ccacaagtcc tgacccccag cccaggaag cagaagctga acagaaagta cagggtcccac 780  
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agcaacatca ctgatcaagc ctaa 1884

<210> 8  
<211> 627  
<212> PRT  
<213> Homo sapiens

<400> 8  
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20 25 30  
Ser Glu Ala Ser Cys Ala Tyr Val Leu Ile Val Thr Ala Val Tyr Trp  
35 40 45  
Val Ser Glu Ala Val Pro Leu Gly Ala Ala Ala Leu Val Pro Ala Phe

50	55	60
Leu Tyr Pro Phe Phe Gly Val Leu Arg Ser Asn Glu Val Ala Ala Glu		
65	70	75
Tyr Phe Lys Asn Thr Thr Leu Leu Leu Val Gly Val Ile Cys Val Ala		80
	85	90
Ala Ala Val Glu Lys Trp Asn Leu His Lys Arg Ile Ala Leu Arg Met		95
	100	105
Val Leu Met Ala Gly Ala Lys Pro Gly Met Leu Leu Leu Cys Phe Met		110
	115	120
Cys Cys Thr Thr Leu Leu Ser Met Trp Leu Ser Asn Thr Ser Thr Thr		125
	130	135
Ala Met Val Met Pro Ile Val Glu Ala Val Leu Gln Glu Leu Val Ser		140
145	150	155
Ala Glu Asp Glu Gln Leu Val Ala Gly Asn Ser Asn Thr Glu Glu Ala		160
	165	170
Glu Pro Ile Ser Leu Asp Val Lys Asn Ser Gln Pro Ser Leu Glu Leu		175
	180	185
Ile Phe Val Asn Glu Asp Arg Ser Asn Ala Asp Leu Thr Thr Leu Met		190
	195	200
His Asn Glu Asn Leu Asn Gly Val Pro Ser Ile Thr Asn Pro Ile Lys		205
	210	215
Thr Ala Asn Gln His Gln Gly Lys Lys Gln His Pro Ser Gln Glu Lys		220
225	230	235
Pro Gln Val Leu Thr Pro Ser Pro Arg Lys Gln Lys Leu Asn Arg Lys		240
	245	250
Tyr Arg Ser His His Asp Gln Met Ile Cys Lys Cys Leu Ser Leu Ser		255
	260	265
Ile Ser Tyr Ser Ala Thr Ile Gly Gly Leu Thr Thr Ile Ile Gly Thr		270
	275	280
Ser Thr Ser Leu Ile Phe Leu Glu His Phe Asn Asn Gln Tyr Pro Ala		285
	290	295
Ala Glu Val Val Asn Phe Gly Thr Trp Phe Leu Phe Ser Phe Pro Ile		300
305	310	315
Ser Leu Ile Met Leu Val Val Ser Trp Phe Trp Met His Trp Leu Phe		320
	325	330
Leu Gly Cys Asn Phe Lys Glu Thr Cys Ser Leu Ser Lys Lys Lys Lys		335
	340	345
Thr Lys Arg Glu Gln Leu Ser Glu Lys Arg Ile Gln Glu Tyr Glu		350
	355	360
Lys Leu Gly Asp Ile Ser Tyr Pro Glu Met Val Thr Gly Phe Phe Phe		365
	370	375
Ile Leu Met Thr Val Leu Trp Phe Thr Arg Glu Pro Gly Phe Val Pro		380
385	390	395
Gly Trp Asp Ser Phe Phe Glu Lys Lys Gly Tyr Arg Thr Asp Ala Thr		400
	405	410
Val Ser Val Phe Leu Gly Phe Leu Leu Phe Leu Ile Pro Ala Lys Lys		415
	420	425
Pro Cys Phe Gly Lys Lys Asn Asp Gly Glu Asn Gln Glu His Ser Leu		430
	435	440
Gly Thr Glu Ser Ile Ile Thr Trp Lys Asp Phe Gln Lys Thr Met Pro		445
	450	455
Trp Glu Ile Val Ile Leu Val Gly Gly Gly Tyr Ala Leu Ala Ser Gly		460
465	470	475
Ser Lys Ser Ser Gly Leu Ser Thr Trp Ile Gly Asn Gln Met Leu Ser		480
	485	490
		495

Leu Ser Ser Leu Pro Pro Trp Ala Val Thr Leu Leu Ala Cys Ile Leu  
 500 505 510  
 Val Ser Ile Val Thr Glu Phe Val Ser Asn Pro Ala Thr Ile Thr Ile  
 515 520 525  
 Phe Leu Pro Ile Leu Cys Ser Leu Ser Glu Thr Leu His Ile Asn Pro  
 530 535 540  
 Leu Tyr Thr Leu Ile Pro Val Thr Met Cys Ile Ser Phe Ala Val Met  
 545 550 555 560  
 Leu Pro Val Gly Asn Pro Pro Asn Ala Ile Val Phe Ser Tyr Gly His  
 565 570 575  
 Cys Gln Ile Lys Asp Met Val Lys Ala Gly Leu Gly Val Asn Val Ile  
 580 585 590  
 Gly Leu Val Ile Val Met Val Ala Ile Asn Thr Trp Gly Val Ser Leu  
 595 600 605  
 Phe His Leu Asp Thr Tyr Pro Ala Trp Ala Arg Val Ser Asn Ile Thr  
 610 615 620  
 Asp Gln Ala  
 625

<210> 9  
 <211> 1884  
 <212> DNA  
 <213> Homo sapiens

<400> 9  
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 aagtggaaac tgcataagcg cattgctctg cgcatggctt tgatggccgg rgccaagccg 360  
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agcaacatca ctgatcaagc ctaa 1884

<210> 10  
<211> 627  
<212> PRT  
<213> Homo sapiens

<400> 10  
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Ser Glu Ala Ser Cys Ala Tyr Val Leu Ile Val Thr Ala Val Tyr Trp  
35 40 45  
Val Ser Glu Ala Val Pro Leu Gly Ala Ala Ala Leu Val Pro Ala Phe  
50 55 60  
Leu Tyr Pro Phe Phe Gly Val Leu Arg Ser Asn Glu Val Ala Ala Glu  
65 70 75 80  
Tyr Phe Lys Asn Thr Thr Leu Leu Leu Val Gly Val Ile Cys Val Ala  
85 90 95  
Ala Ala Val Glu Lys Trp Asn Leu His Lys Arg Ile Ala Leu Arg Met  
100 105 110  
Val Leu Met Ala Gly Ala Lys Pro Gly Met Leu Leu Leu Cys Phe Met  
115 120 125  
Cys Cys Thr Thr Leu Leu Ser Met Trp Leu Ser Asn Thr Ser Thr Thr  
130 135 140  
Ala Met Val Met Pro Ile Val Glu Ala Val Leu Gln Glu Leu Val Ser  
145 150 155 160  
Ala Glu Asp Glu Gln Leu Val Ala Gly Asn Ser Asn Thr Glu Glu Ala  
165 170 175  
Glu Pro Ile Ser Leu Asp Val Lys Asn Ser Gln Pro Ser Leu Glu Leu  
180 185 190  
Ile Phe Val Asn Glu Asp Arg Ser Asn Ala Asp Leu Thr Thr Leu Met  
195 200 205  
His Asn Glu Asn Leu Asn Gly Val Pro Ser Ile Thr Asn Pro Ile Lys  
210 215 220  
Thr Ala Asn Gln His Gln Gly Lys Lys Gln His Pro Ser Gln Glu Lys  
225 230 235 240  
Pro Gln Val Leu Thr Pro Ser Pro Arg Lys Gln Lys Leu Asn Arg Lys  
245 250 255  
Tyr Arg Ser His His Asp Gln Met Ile Cys Lys Cys Leu Ser Leu Ser  
260 265 270  
Ile Ser Tyr Ser Ala Thr Ile Gly Gly Leu Thr Thr Ile Ile Gly Thr  
275 280 285  
Ser Thr Ser Leu Ile Phe Leu Glu His Phe Asn Asn Gln Tyr Pro Ala  
290 295 300  
Ala Glu Val Val Asn Phe Gly Thr Trp Phe Leu Phe Ser Phe Pro Ile  
305 310 315 320  
Ser Leu Ile Met Leu Val Val Ser Trp Phe Trp Met His Trp Leu Phe  
325 330 335  
Leu Gly Cys Asn Phe Lys Glu Thr Cys Ser Leu Ser Lys Lys Lys Lys  
340 345 350  
Thr Lys Arg Glu Gln Leu Ser Glu Lys Arg Ile Gln Glu Glu Tyr Glu  
355 360 365

Lys Leu Gly Asp Ile Ser Tyr Pro Glu Met Val Thr Gly Phe Phe Phe  
 370 375 380  
 Ile Leu Met Thr Val Leu Trp Phe Thr Arg Glu Pro Gly Phe Val Pro  
 385 390 395 400  
 Gly Trp Asp Ser Phe Phe Glu Lys Lys Gly Tyr Arg Thr Asp Ala Thr  
 405 410 415  
 Val Ser Val Phe Leu Gly Phe Leu Leu Phe Leu Ile Pro Ala Lys Lys  
 420 425 430  
 Pro Cys Phe Gly Lys Lys Asn Asp Gly Glu Asn Gln Glu His Ser Leu  
 435 440 445  
 Gly Thr Glu Ser Ile Ile Thr Trp Lys Asp Phe Gln Lys Thr Met Pro  
 450 455 460  
 Trp Glu Ile Val Ile Leu Val Gly Gly Gly Tyr Ala Leu Ala Ser Gly  
 465 470 475 480  
 Ser Lys Ser Ser Gly Leu Ser Thr Trp Ile Gly Asn Gln Met Leu Ser  
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 Val Ser Ile Val Thr Glu Phe Val Ser Asn Pro Ala Thr Ile Thr Ile  
 515 520 525  
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 530 535 540  
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 565 570 575  
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 580 585 590  
 Gly Leu Val Ile Val Met Val Ala Ile Asn Thr Trp Gly Val Ser Leu  
 595 600 605  
 Phe His Leu Asp Thr Tyr Pro Ala Trp Ala Arg Val Ser Asn Ile Thr  
 610 615 620  
 Asp Gln Ala  
 625

&lt;210&gt; 11

&lt;211&gt; 1746

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 11

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aagaggatcc aagaagaata tgaaaaactg ggagacatta gctaccaga aatggtgact   1140
ggatttttct tcctcctgat gaccgtactg tggtttaccg gggagcctgg ctttgtccct   1200
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ggagagaacc aggagcactc actggggacc gagtccatca tcacgtggaa ggacttccag   1380
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ccaccgtggg ctgtcaccct gctggcatgc atcctcgtgt ccattgtcac tgagtttgtg   1560
agcaaccag caaccatcac catcttcctg cccatcctgt gcagcctggt gaaagctggc   1620
ctgggagtc aagttattgg actgggtgata gtaatggtgg ccatcaacac ctggggagtt   1680
agcctcttcc acctggacac ttaccagca tgggcgaggg tcagcaacat cactgatcaa   1740
gcctaa                                1746

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&lt;210&gt; 12

&lt;211&gt; 581

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 12

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Met Gly Leu Leu Gln Gly Leu Leu Arg Val Arg Lys Leu Leu Leu Val
 1              5              10              15
Val Cys Val Pro Leu Leu Leu Leu Pro Leu Pro Val Leu His Pro Ser
      20              25              30
Ser Glu Ala Ser Cys Ala Tyr Val Leu Ile Val Thr Ala Val Tyr Trp
      35              40              45
Val Ser Glu Ala Val Pro Leu Gly Ala Ala Ala Leu Val Pro Ala Phe
      50              55              60
Leu Tyr Pro Phe Phe Gly Val Leu Arg Ser Asn Glu Val Ala Ala Glu
65              70              75              80
Tyr Phe Lys Asn Thr Thr Leu Leu Leu Val Gly Val Ile Cys Val Ala
      85              90              95
Ala Ala Val Glu Lys Trp Asn Leu His Lys Arg Ile Ala Leu Arg Met
      100             105             110
Val Leu Met Ala Gly Ala Lys Pro Gly Met Leu Leu Leu Cys Phe Met
      115             120             125
Cys Cys Thr Thr Leu Leu Ser Met Trp Leu Ser Asn Thr Ser Thr Thr
      130             135             140
Ala Met Val Met Pro Ile Val Glu Ala Val Leu Gln Glu Leu Val Ser
145             150             155             160
Ala Glu Asp Glu Gln Leu Val Ala Gly Asn Ser Asn Thr Glu Glu Ala
      165             170             175
Glu Pro Ile Ser Leu Asp Val Lys Asn Ser Gln Pro Ser Leu Glu Leu
      180             185             190
Ile Phe Val Asn Glu Asp Arg Ser Asn Ala Asp Leu Thr Thr Leu Met
      195             200             205
His Asn Glu Asn Leu Asn Gly Val Pro Ser Ile Thr Asn Pro Ile Lys
      210             215             220
Thr Ala Asn Gln His Gln Gly Lys Lys Gln His Pro Ser Gln Glu Lys
225             230             235             240
Pro Gln Val Leu Thr Pro Ser Pro Arg Lys Gln Lys Leu Asn Arg Lys
      245             250             255

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Tyr Arg Ser His His Asp Gln Met Ile Cys Lys Cys Leu Ser Leu Ser  
 260 265 270  
 Ile Ser Tyr Ser Ala Thr Ile Gly Gly Leu Thr Thr Ile Ile Gly Thr  
 275 280 285  
 Ser Thr Ser Leu Ile Phe Leu Glu His Phe Asn Asn Gln Tyr Pro Ala  
 290 295 300  
 Ala Glu Val Val Asn Phe Gly Thr Trp Phe Leu Phe Ser Phe Pro Ile  
 305 310 315 320  
 Ser Leu Ile Met Leu Val Val Ser Trp Phe Trp Met His Trp Leu Phe  
 325 330 335  
 Leu Gly Cys Asn Phe Lys Glu Thr Cys Ser Leu Ser Lys Lys Lys Lys  
 340 345 350  
 Thr Lys Arg Glu Gln Leu Ser Glu Lys Arg Ile Gln Glu Glu Tyr Glu  
 355 360 365  
 Lys Leu Gly Asp Ile Ser Tyr Pro Glu Met Val Thr Gly Phe Phe Phe  
 370 375 380  
 Ile Leu Met Thr Val Leu Trp Phe Thr Arg Glu Pro Gly Phe Val Pro  
 385 390 395 400  
 Gly Trp Asp Ser Phe Phe Glu Lys Lys Gly Tyr Arg Thr Asp Ala Thr  
 405 410 415  
 Val Ser Val Phe Leu Gly Phe Leu Leu Phe Leu Ile Pro Ala Lys Lys  
 420 425 430  
 Pro Cys Phe Gly Lys Lys Asn Asp Gly Glu Asn Gln Glu His Ser Leu  
 435 440 445  
 Gly Thr Glu Ser Ile Ile Thr Trp Lys Asp Phe Gln Lys Thr Met Pro  
 450 455 460  
 Trp Glu Ile Val Ile Leu Val Gly Gly Gly Tyr Ala Leu Ala Ser Gly  
 465 470 475 480  
 Ser Lys Ser Ser Gly Leu Ser Thr Trp Ile Gly Asn Gln Met Leu Ser  
 485 490 495  
 Leu Ser Ser Leu Pro Pro Trp Ala Val Thr Leu Leu Ala Cys Ile Leu  
 500 505 510  
 Val Ser Ile Val Thr Glu Phe Val Ser Asn Pro Ala Thr Ile Thr Ile  
 515 520 525  
 Phe Leu Pro Ile Leu Cys Ser Leu Val Lys Ala Gly Leu Gly Val Asn  
 530 535 540  
 Val Ile Gly Leu Val Ile Val Met Val Ala Ile Asn Thr Trp Gly Val  
 545 550 555 560  
 Ser Leu Phe His Leu Asp Thr Tyr Pro Ala Trp Ala Arg Val Ser Asn  
 565 570 575  
 Ile Thr Asp Gln Ala  
 580

&lt;210&gt; 13

&lt;211&gt; 1881

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 13

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ctgatcgtga ctgctgtgta ctgggtgtcg gaggcagtc ctctgggagc tgcagccctg	180
gtgccggcct tcctytaccc gttcttcgga gtccctccggt ccaatgaggt ggcggcgagag	240
tacttcaaga acaccacgct gctgctggtg ggggtcatct gcgtggcggc tgccgtggag	300
aagtgggaacc tgcataagcg cattgctctg cgcattggtct tgatggcccg rgccaagccg	360

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aacacctggg gagttagcct cttccacctg gacacttacc cagcatgggc gagggtcagc 1860
aacatcactg atcaagccta a 1881

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&lt;210&gt; 14

&lt;211&gt; 626

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 14

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Met Gly Leu Leu Gln Gly Leu Leu Arg Val Arg Lys Leu Leu Leu Val
1           5           10           15
Val Cys Val Pro Leu Leu Leu Leu Pro Leu Pro Val Leu His Pro Ser
20           25           30
Ser Glu Ala Ser Cys Ala Tyr Val Leu Ile Val Thr Ala Val Tyr Trp
35           40           45
Val Ser Glu Ala Val Pro Leu Gly Ala Ala Ala Leu Val Pro Ala Phe
50           55           60
Leu Tyr Pro Phe Phe Gly Val Leu Arg Ser Asn Glu Val Ala Ala Glu
65           70           75           80
Tyr Phe Lys Asn Thr Thr Leu Leu Leu Val Gly Val Ile Cys Val Ala
85           90           95
Ala Ala Val Glu Lys Trp Asn Leu His Lys Arg Ile Ala Leu Arg Met
100          105          110
Val Leu Met Ala Gly Ala Lys Pro Gly Met Leu Leu Leu Cys Phe Met
115          120          125
Cys Cys Thr Thr Leu Leu Ser Met Trp Leu Ser Asn Thr Ser Thr Thr
130          135          140
Ala Met Val Met Pro Ile Val Glu Ala Val Leu Gln Glu Leu Val Ser
145          150          155          160
Ala Glu Asp Glu Gln Leu Val Ala Gly Asn Ser Asn Thr Glu Glu Ala
165          170          175

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Glu Pro Ile Ser Leu Asp Val Lys Asn Ser Gln Pro Ser Leu Glu Leu  
 180 185 190  
 Ile Phe Val Asn Glu Glu Ser Asn Ala Asp Leu Thr Thr Leu Met His  
 195 200 205  
 Asn Glu Asn Leu Asn Gly Val Pro Ser Ile Thr Asn Pro Ile Lys Thr  
 210 215 220  
 Ala Asn Gln His Gln Gly Lys Lys Gln His Pro Ser Gln Glu Lys Pro  
 225 230 235 240  
 Gln Val Leu Thr Pro Ser Pro Arg Lys Gln Lys Leu Asn Arg Lys Tyr  
 245 250 255  
 Arg Ser His His Asp Gln Met Ile Cys Lys Cys Leu Ser Leu Ser Ile  
 260 265 270  
 Ser Tyr Ser Ala Thr Ile Gly Gly Leu Thr Thr Ile Ile Gly Thr Ser  
 275 280 285  
 Thr Ser Leu Ile Phe Leu Glu His Phe Asn Asn Gln Tyr Pro Ala Ala  
 290 295 300  
 Glu Val Val Asn Phe Gly Thr Trp Phe Leu Phe Ser Phe Pro Ile Ser  
 305 310 315 320  
 Leu Ile Met Leu Val Val Ser Trp Phe Trp Met His Trp Leu Phe Leu  
 325 330 335  
 Gly Cys Asn Phe Lys Glu Thr Cys Ser Leu Ser Lys Lys Lys Lys Thr  
 340 345 350  
 Lys Arg Glu Gln Leu Ser Glu Lys Arg Ile Gln Glu Glu Tyr Glu Lys  
 355 360 365  
 Leu Gly Asp Ile Ser Tyr Pro Glu Met Val Thr Gly Phe Phe Phe Ile  
 370 375 380  
 Leu Met Thr Val Leu Trp Phe Thr Arg Glu Pro Gly Phe Val Pro Gly  
 385 390 395 400  
 Trp Asp Ser Phe Phe Glu Lys Lys Gly Tyr Arg Thr Asp Ala Thr Val  
 405 410 415  
 Ser Val Phe Leu Gly Phe Leu Leu Phe Leu Ile Pro Ala Lys Lys Pro  
 420 425 430  
 Cys Phe Gly Lys Lys Asn Asp Gly Glu Asn Gln Glu His Ser Leu Gly  
 435 440 445  
 Thr Glu Pro Ile Ile Thr Trp Lys Asp Phe Gln Lys Thr Met Pro Trp  
 450 455 460  
 Glu Ile Val Ile Leu Val Gly Gly Gly Tyr Ala Leu Ala Ser Gly Ser  
 465 470 475 480  
 Lys Ser Ser Gly Leu Ser Thr Trp Ile Gly Asn Gln Met Leu Ser Leu  
 485 490 495  
 Ser Ser Leu Pro Pro Trp Ala Val Thr Leu Leu Ala Cys Ile Leu Val  
 500 505 510  
 Ser Ile Val Thr Glu Phe Val Ser Asn Pro Ala Thr Ile Thr Ile Phe  
 515 520 525  
 Leu Pro Ile Leu Cys Ser Leu Ser Glu Thr Leu His Ile Asn Pro Leu  
 530 535 540  
 Tyr Thr Leu Ile Pro Val Thr Met Cys Ile Ser Phe Ala Val Met Leu  
 545 550 555 560  
 Pro Val Gly Asn Pro Pro Asn Ala Ile Val Phe Ser Tyr Gly His Cys  
 565 570 575  
 Gln Ile Lys Asp Met Val Lys Ala Gly Leu Gly Val Asn Val Ile Gly  
 580 585 590  
 Leu Val Ile Val Met Val Ala Ile Asn Thr Trp Gly Val Ser Leu Phe  
 595 600 605  
 His Leu Asp Thr Tyr Pro Ala Trp Ala Arg Val Ser Asn Ile Thr Asp

610  
Gln Ala  
625

615

620

<210> 15  
<211> 1881  
<212> DNA  
<213> Homo sapiens

&lt;400&gt; 15

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aagtggaaac	tgcataagcg	cattgctctg	cgcatgggtc	tgatggccgg	rgccaagccg	360
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aacacctggg	gagttagcct	cttccacctg	gacacttacc	cagcatgggc	gagggtcagc	1860
aacatcactg	atcaagccta	a				1881

<210> 16  
<211> 626  
<212> PRT  
<213> Homo sapiens

&lt;400&gt; 16

Met	Gly	Leu	Leu	Gln	Gly	Leu	Leu	Arg	Val	Arg	Lys	Leu	Leu	Leu	Val
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Val	Cys	Val	Pro	Leu	Leu	Leu	Leu	Pro	Leu	Pro	Val	Leu	His	Pro	Ser
			20					25				30			
Ser	Glu	Ala	Ser	Cys	Ala	Tyr	Val	Leu	Ile	Val	Thr	Ala	Val	Tyr	Trp
		35					40				45				

Val Ser Glu Ala Val Pro Leu Gly Ala Ala Ala Leu Val Pro Ala Phe  
 50 55 60  
 Leu Tyr Pro Phe Phe Gly Val Leu Arg Ser Asn Glu Val Ala Ala Glu  
 65 70 75 80  
 Tyr Phe Lys Asn Thr Thr Leu Leu Leu Val Gly Val Ile Cys Val Ala  
 85 90 95  
 Ala Ala Val Glu Lys Trp Asn Leu His Lys Arg Ile Ala Leu Arg Met  
 100 105 110  
 Val Leu Met Ala Gly Ala Lys Pro Gly Met Leu Leu Leu Cys Phe Met  
 115 120 125  
 Cys Cys Thr Thr Leu Leu Ser Met Trp Leu Ser Asn Thr Ser Thr Thr  
 130 135 140  
 Ala Met Val Met Pro Ile Val Glu Ala Val Leu Gln Glu Leu Val Ser  
 145 150 155 160  
 Ala Glu Asp Glu Gln Leu Val Ala Gly Asn Ser Asn Thr Glu Glu Ala  
 165 170 175  
 Glu Pro Ile Ser Leu Asp Val Lys Asn Ser Gln Pro Ser Leu Glu Leu  
 180 185 190  
 Ile Phe Val Asn Glu Glu Ser Asn Ala Asp Leu Thr Thr Leu Met His  
 195 200 205  
 Asn Glu Asn Leu Asn Gly Val Pro Ser Ile Thr Asn Pro Ile Lys Thr  
 210 215 220  
 Ala Asn Gln His Gln Gly Lys Lys Gln His Pro Ser Gln Glu Lys Pro  
 225 230 235 240  
 Gln Val Leu Thr Pro Ser Pro Arg Lys Gln Lys Leu Asn Arg Lys Tyr  
 245 250 255  
 Arg Ser His His Asp Gln Met Ile Cys Lys Cys Leu Ser Leu Ser Ile  
 260 265 270  
 Ser Tyr Ser Ala Thr Ile Gly Gly Leu Thr Thr Ile Ile Gly Thr Ser  
 275 280 285  
 Thr Ser Leu Ile Phe Leu Glu His Phe Asn Asn Gln Tyr Pro Ala Ala  
 290 295 300  
 Glu Val Val Asn Phe Gly Thr Trp Phe Leu Phe Ser Phe Pro Ile Ser  
 305 310 315 320  
 Leu Ile Met Leu Val Val Ser Trp Phe Trp Met His Trp Leu Phe Leu  
 325 330 335  
 Gly Cys Asn Phe Lys Glu Thr Cys Ser Leu Ser Lys Lys Lys Lys Thr  
 340 345 350  
 Lys Arg Glu Gln Leu Ser Glu Lys Arg Ile Gln Glu Glu Tyr Glu Lys  
 355 360 365  
 Leu Gly Asp Ile Ser Tyr Pro Glu Met Val Thr Gly Phe Phe Phe Ile  
 370 375 380  
 Leu Met Thr Val Leu Trp Phe Thr Arg Glu Pro Gly Phe Val Pro Gly  
 385 390 395 400  
 Trp Asp Ser Phe Phe Glu Lys Lys Gly Tyr Arg Thr Asp Ala Thr Val  
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 Ser Val Phe Leu Gly Phe Leu Leu Phe Leu Ile Pro Ala Lys Lys Pro  
 420 425 430  
 Cys Phe Gly Lys Lys Asn Asp Gly Glu Asn Gln Glu His Ser Leu Gly  
 435 440 445  
 Thr Glu Pro Ile Ile Thr Trp Lys Asp Phe Gln Lys Thr Met Pro Trp  
 450 455 460  
 Glu Ile Val Ile Leu Val Gly Gly Gly Tyr Ala Leu Ala Ser Gly Ser  
 465 470 475 480  
 Lys Ser Ser Gly Leu Ser Thr Trp Ile Gly Asn Gln Met Leu Ser Leu

	485		490		495
Ser Ser Leu Pro Pro Trp Ala Val Thr Leu Leu Ala Cys Ile Leu Val					
	500		505		510
Ser Ile Val Thr Glu Phe Val Ser Asn Pro Ala Thr Ile Thr Ile Phe					
	515		520		525
Leu Pro Ile Leu Cys Ser Leu Ser Glu Thr Gln His Ile Asn Pro Leu					
	530		535		540
Tyr Thr Leu Ile Pro Val Thr Met Cys Ile Ser Phe Ala Val Met Leu					
545		550		555	560
Pro Val Gly Asn Pro Pro Asn Ala Ile Val Phe Ser Tyr Gly His Cys					
	565		570		575
Gln Ile Lys Asp Met Val Lys Ala Gly Leu Gly Val Asn Val Ile Gly					
	580		585		590
Leu Val Ile Val Met Val Ala Ile Asn Thr Trp Gly Val Ser Leu Phe					
	595		600		605
His Leu Asp Thr Tyr Pro Ala Trp Ala Arg Val Ser Asn Ile Thr Asp					
	610		615		620
Gln Ala					
625					

&lt;210&gt; 17

&lt;211&gt; 1743

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 17

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taa

1743

<210> 18  
 <211> 580  
 <212> PRT  
 <213> Homo sapiens

<400> 18  
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 20 25 30  
 Ser Glu Ala Ser Cys Ala Tyr Val Leu Ile Val Thr Ala Val Tyr Trp  
 35 40 45  
 Val Ser Glu Ala Val Pro Leu Gly Ala Ala Ala Leu Val Pro Ala Phe  
 50 55 60  
 Leu Tyr Pro Phe Phe Gly Val Leu Arg Ser Asn Glu Val Ala Ala Glu  
 65 70 75 80  
 Tyr Phe Lys Asn Thr Thr Leu Leu Leu Val Gly Val Ile Cys Val Ala  
 85 90 95  
 Ala Ala Val Glu Lys Trp Asn Leu His Lys Arg Ile Ala Leu Arg Met  
 100 105 110  
 Val Leu Met Ala Gly Ala Lys Pro Gly Met Leu Leu Leu Cys Phe Met  
 115 120 125  
 Cys Cys Thr Thr Leu Leu Ser Met Trp Leu Ser Asn Thr Ser Thr Thr  
 130 135 140  
 Ala Met Val Met Pro Ile Val Glu Ala Val Leu Gln Glu Leu Val Ser  
 145 150 155 160  
 Ala Glu Asp Glu Gln Leu Val Ala Gly Asn Ser Asn Thr Glu Glu Ala  
 165 170 175  
 Glu Pro Ile Ser Leu Asp Val Lys Asn Ser Gln Pro Ser Leu Glu Leu  
 180 185 190  
 Ile Phe Val Asn Glu Glu Ser Asn Ala Asp Leu Thr Thr Leu Met His  
 195 200 205  
 Asn Glu Asn Leu Asn Gly Val Pro Ser Ile Thr Asn Pro Ile Lys Thr  
 210 215 220  
 Ala Asn Gln His Gln Gly Lys Lys Gln His Pro Ser Gln Glu Lys Pro  
 225 230 235 240  
 Gln Val Leu Thr Pro Ser Pro Arg Lys Gln Lys Leu Asn Arg Lys Tyr  
 245 250 255  
 Arg Ser His His Asp Gln Met Ile Cys Lys Cys Leu Ser Leu Ser Ile  
 260 265 270  
 Ser Tyr Ser Ala Thr Ile Gly Gly Leu Thr Thr Ile Ile Gly Thr Ser  
 275 280 285  
 Thr Ser Leu Ile Phe Leu Glu His Phe Asn Asn Gln Tyr Pro Ala Ala  
 290 295 300  
 Glu Val Val Asn Phe Gly Thr Trp Phe Leu Phe Ser Phe Pro Ile Ser  
 305 310 315 320  
 Leu Ile Met Leu Val Val Ser Trp Phe Trp Met His Trp Leu Phe Leu  
 325 330 335  
 Gly Cys Asn Phe Lys Glu Thr Cys Ser Leu Ser Lys Lys Lys Lys Thr  
 340 345 350  
 Lys Arg Glu Gln Leu Ser Glu Lys Arg Ile Gln Glu Glu Tyr Glu Lys  
 355 360 365  
 Leu Gly Asp Ile Ser Tyr Pro Glu Met Val Thr Gly Phe Phe Phe Ile

370 375 380  
 Leu Met Thr Val Leu Trp Phe Thr Arg Glu Pro Gly Phe Val Pro Gly  
 385 390 395 400  
 Trp Asp Ser Phe Phe Glu Lys Lys Gly Tyr Arg Thr Asp Ala Thr Val  
 405 410 415  
 Ser Val Phe Leu Gly Phe Leu Leu Phe Leu Ile Pro Ala Lys Lys Pro  
 420 425 430  
 Cys Phe Gly Lys Lys Asn Asp Gly Glu Asn Gln Glu His Ser Leu Gly  
 435 440 445  
 Thr Glu Pro Ile Ile Thr Trp Lys Asp Phe Gln Lys Thr Met Pro Trp  
 450 455 460  
 Glu Ile Val Ile Leu Val Gly Gly Gly Tyr Ala Leu Ala Ser Gly Ser  
 465 470 475 480  
 Lys Ser Ser Gly Leu Ser Thr Trp Ile Gly Asn Gln Met Leu Ser Leu  
 485 490 495  
 Ser Ser Leu Pro Pro Trp Ala Val Thr Leu Leu Ala Cys Ile Leu Val  
 500 505 510  
 Ser Ile Val Thr Glu Phe Val Ser Asn Pro Ala Thr Ile Thr Ile Phe  
 515 520 525  
 Leu Pro Ile Leu Cys Ser Leu Val Lys Ala Gly Leu Gly Val Asn Val  
 530 535 540  
 Ile Gly Leu Val Ile Val Met Val Ala Ile Asn Thr Trp Gly Val Ser  
 545 550 555 560  
 Leu Phe His Leu Asp Thr Tyr Pro Ala Trp Ala Arg Val Ser Asn Ile  
 565 570 575  
 Thr Asp Gln Ala  
 580

&lt;210&gt; 19

&lt;211&gt; 1881

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 19

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gtgccggcct	tcctytaccc	gttcttcgga	gtcctccggt	ccaatgaggt	ggcggcggag	240
tacttcaaga	acaccacgct	gctgctgggtg	gggggtcatct	gcgtggcggc	tgccgtggag	300
aagtggaacc	tgcataagcg	cattgctctg	cgcatggctct	tgatggccgg	rgccaagccg	360
ggcatgctgc	tgctctgctt	catgtgctgt	accacgttgc	tgtccatgtg	gctgtccaac	420
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gctgaggacg	agcagctcgt	ggcgggcaac	tccaacaccg	aagaggccga	acccatcagt	540
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cccatcaaaa	ctgcaaacca	acaccagggc	aagaagcaac	acccatccca	ggaaaagcca	720
caagtctga	ccccagccc	caggaagcag	aagctgaaca	gaaagtacag	gtcccacat	780
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aacatcactg atcaagccta a 1881

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&lt;210&gt; 20

&lt;211&gt; 626

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 20

```

Met Gly Leu Leu Gln Gly Leu Leu Arg Val Arg Lys Leu/Leu Leu Val
1           5           10          15
Val Cys Val Pro Leu Leu Leu Leu Pro Leu Pro Val Leu His Pro Ser
20          25          30
Ser Glu Ala Ser Cys Ala Tyr Val Leu Ile Val Thr Ala Val Tyr Trp
35          40          45
Val Ser Glu Ala Val Pro Leu Gly Ala Ala Ala Leu Val Pro Ala Phe
50          55          60
Leu Tyr Pro Phe Phe Gly Val Leu Arg Ser Asn Glu Val Ala Ala Glu
65          70          75          80
Tyr Phe Lys Asn Thr Thr Leu Leu Leu Val Gly Val Ile Cys Val Ala
85          90          95
Ala Ala Val Glu Lys Trp Asn Leu His Lys Arg Ile Ala Leu Arg Met
100         105         110
Val Leu Met Ala Gly Ala Lys Pro Gly Met Leu Leu Leu Cys Phe Met
115         120         125
Cys Cys Thr Thr Leu Leu Ser Met Trp Leu Ser Asn Thr Ser Thr Thr
130         135         140
Ala Met Val Met Pro Ile Val Glu Ala Val Leu Gln Glu Leu Val Ser
145         150         155         160
Ala Glu Asp Glu Gln Leu Val Ala Gly Asn Ser Asn Thr Glu Glu Ala
165         170         175
Glu Pro Ile Ser Leu Asp Val Lys Asn Ser Gln Pro Ser Leu Glu Leu
180         185         190
Ile Phe Val Asn Glu Glu Ser Asn Ala Asp Leu Thr Thr Leu Met His
195         200         205
Asn Glu Asn Leu Asn Gly Val Pro Ser Ile Thr Asn Pro Ile Lys Thr
210         215         220
Ala Asn Gln His Gln Gly Lys Lys Gln His Pro Ser Gln Glu Lys Pro
225         230         235         240
Gln Val Leu Thr Pro Ser Pro Arg Lys Gln Lys Leu Asn Arg Lys Tyr
245         250         255
Arg Ser His His Asp Gln Met Ile Cys Lys Cys Leu Ser Leu Ser Ile
260         265         270
Ser Tyr Ser Ala Thr Ile Gly Gly Leu Thr Thr Ile Ile Gly Thr Ser
275         280         285
Thr Ser Leu Ile Phe Leu Glu His Phe Asn Asn Gln Tyr Pro Ala Ala

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290 295 300  
 Glu Val Val Asn Phe Gly Thr Trp Phe Leu Phe Ser Phe Pro Ile Ser  
 305 310 315 320  
 Leu Ile Met Leu Val Ser Trp Phe Trp Met His Trp Leu Phe Leu  
 325 330 335  
 Gly Cys Asn Phe Lys Glu Thr Cys Ser Leu Ser Lys Lys Lys Lys Thr  
 340 345 350  
 Lys Arg Glu Gln Leu Ser Glu Lys Arg Ile Gln Glu Glu Tyr Glu Lys  
 355 360 365  
 Leu Gly Asp Ile Ser Tyr Pro Glu Met Val Thr Gly Phe Phe Phe Ile  
 370 375 380  
 Leu Met Thr Val Leu Trp Phe Thr Arg Glu Pro Gly Phe Val Pro Gly  
 385 390 395 400  
 Trp Asp Ser Phe Phe Glu Lys Lys Gly Tyr Arg Thr Asp Ala Thr Val  
 405 410 415  
 Ser Val Phe Leu Gly Phe Leu Leu Phe Leu Ile Pro Ala Lys Lys Pro  
 420 425 430  
 Cys Phe Gly Lys Lys Asn Asp Gly Glu Asn Gln Glu His Ser Leu Gly  
 435 440 445  
 Thr Glu Ser Ile Ile Thr Trp Lys Asp Phe Gln Lys Thr Met Pro Trp  
 450 455 460  
 Glu Ile Val Ile Leu Val Gly Gly Gly Tyr Ala Leu Ala Ser Gly Ser  
 465 470 475 480  
 Lys Ser Ser Gly Leu Ser Thr Trp Ile Gly Asn Gln Met Leu Ser Leu  
 485 490 495  
 Ser Ser Leu Pro Pro Trp Ala Val Thr Leu Leu Ala Cys Ile Leu Val  
 500 505 510  
 Ser Ile Val Thr Glu Phe Val Ser Asn Pro Ala Thr Ile Thr Ile Phe  
 515 520 525  
 Leu Pro Ile Leu Cys Ser Leu Ser Glu Thr Leu His Ile Asn Pro Leu  
 530 535 540  
 Tyr Thr Leu Ile Pro Val Thr Met Cys Ile Ser Phe Ala Val Met Leu  
 545 550 555 560  
 Pro Val Gly Asn Pro Pro Asn Ala Ile Val Phe Ser Tyr Gly His Cys  
 565 570 575  
 Gln Ile Lys Asp Met Val Lys Ala Gly Leu Gly Val Asn Val Ile Gly  
 580 585 590  
 Leu Val Ile Val Met Val Ala Ile Asn Thr Trp Gly Val Ser Leu Phe  
 595 600 605  
 His Leu Asp Thr Tyr Pro Ala Trp Ala Arg Val Ser Asn Ile Thr Asp  
 610 615 620  
 Gln Ala  
 625

&lt;210&gt; 21

&lt;211&gt; 1881

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 21

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 ctgatcgtga ctgctgtgta ctgggtgtcg gaggcagtgc ctctgggagc tgcagccctg 180  
 gtgccggcct tctytaccc gttcttcgga gtcctccggt ccaatgaggt ggcggcggag 240  
 tacttcaaga acaccacgct gctgctggtg ggggtcatct gcgtggcggc tgccgtggag 300

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aagtggaacc tgcataagcg cattgctctg cgcattggtct tgatggccgg rgccaagccg 360
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acctccacca ccgccatggt gatgcccac gtggaggccg tgctgcagga gctggtcagt 480
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gcagacctca cactctgat gcacaacgag aacctgaatg gtgtgccctc gatcaccaac 660
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caagtcctga ccccragccc caggaagcag aagctgaaca gaaagtacag gtcccaccat 780
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aacacctggg gagttagcct cttccacctg gacacttacc cagcatgggc gagggtcagc 1860
aacatcactg atcaagccta a 1881

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&lt;210&gt; 22

&lt;211&gt; 626

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 22

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Met Gly Leu Leu Gln Gly Leu Leu Arg Val Arg Lys Leu Leu Leu Val
1          5          10          15
Val Cys Val Pro Leu Leu Leu Leu Pro Leu Pro Val Leu His Pro Ser
20          25          30
Ser Glu Ala Ser Cys Ala Tyr Val Leu Ile Val Thr Ala Val Tyr Trp
35          40          45
Val Ser Glu Ala Val Pro Leu Gly Ala Ala Ala Leu Val Pro Ala Phe
50          55          60
Leu Tyr Pro Phe Phe Gly Val Leu Arg Ser Asn Glu Val Ala Ala Glu
65          70          75          80
Tyr Phe Lys Asn Thr Thr Leu Leu Leu Val Gly Val Ile Cys Val Ala
85          90          95
Ala Ala Val Glu Lys Trp Asn Leu His Lys Arg Ile Ala Leu Arg Met
100         105         110
Val Leu Met Ala Gly Ala Lys Pro Gly Met Leu Leu Leu Cys Phe Met
115         120         125
Cys Cys Thr Thr Leu Leu Ser Met Trp Leu Ser Asn Thr Ser Thr Thr
130         135         140
Ala Met Val Met Pro Ile Val Glu Ala Val Leu Gln Glu Leu Val Ser
145         150         155         160
Ala Glu Asp Glu Gln Leu Val Ala Gly Asn Ser Asn Thr Glu Glu Ala

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25

His Leu Asp Thr Tyr Pro Ala Trp Ala Arg Val Ser Asn Ile Thr Asp  
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 Gln Ala  
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<210> 23  
 <211> 1743  
 <212> DNA  
 <213> Homo sapiens

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 aagtgaacc tgcataagcg cattgctctg cgcattggtct tgatggcggg rgccaagccg 360  
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 taa 1743

<210> 24  
 <211> 580  
 <212> PRT  
 <213> Homo sapiens

<400> 24  
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 Ser Glu Ala Ser Cys Ala Tyr Val Leu Ile Val Thr Ala Val Tyr Trp  
 35 40 45  
 Val Ser Glu Ala Val Pro Leu Gly Ala Ala Ala Leu Val Pro Ala Phe

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Leu Tyr Pro Phe Phe Gly Val Leu Arg Ser Asn Glu Val Ala Ala Glu		
65	70	75
Tyr Phe Lys Asn Thr Thr Leu Leu Leu Val Gly Val Ile Cys Val Ala		80
	85	90
Ala Ala Val Glu Lys Trp Asn Leu His Lys Arg Ile Ala Leu Arg Met		95
	100	105
Val Leu Met Ala Gly Ala Lys Pro Gly Met Leu Leu Leu Cys Phe Met		110
	115	120
Cys Cys Thr Thr Leu Leu Ser Met Trp Leu Ser Asn Thr Ser Thr Thr		125
	130	135
Ala Met Val Met Pro Ile Val Glu Ala Val Leu Gln Glu Leu Val Ser		140
145	150	155
Ala Glu Asp Glu Gln Leu Val Ala Gly Asn Ser Asn Thr Glu Glu Ala		160
	165	170
Glu Pro Ile Ser Leu Asp Val Lys Asn Ser Gln Pro Ser Leu Glu Leu		175
	180	185
Ile Phe Val Asn Glu Glu Ser Asn Ala Asp Leu Thr Thr Leu Met His		190
	195	200
Asn Glu Asn Leu Asn Gly Val Pro Ser Ile Thr Asn Pro Ile Lys Thr		205
	210	215
Ala Asn Gln His Gln Gly Lys Lys Gln His Pro Ser Gln Glu Lys Pro		220
225	230	235
Gln Val Leu Thr Pro Ser Pro Arg Lys Gln Lys Leu Asn Arg Lys Tyr		240
	245	250
Arg Ser His His Asp Gln Met Ile Cys Lys Cys Leu Ser Leu Ser Ile		255
	260	265
Ser Tyr Ser Ala Thr Ile Gly Gly Leu Thr Thr Ile Ile Gly Thr Ser		270
	275	280
Thr Ser Leu Ile Phe Leu Glu His Phe Asn Asn Gln Tyr Pro Ala Ala		285
	290	295
Glu Val Val Asn Phe Gly Thr Trp Phe Leu Phe Ser Phe Pro Ile Ser		300
305	310	315
Leu Ile Met Leu Val Val Ser Trp Phe Trp Met His Trp Leu Phe Leu		320
	325	330
Gly Cys Asn Phe Lys Glu Thr Cys Ser Leu Ser Lys Lys Lys Lys Thr		335
	340	345
Lys Arg Glu Gln Leu Ser Glu Lys Arg Ile Gln Glu Glu Tyr Glu Lys		350
	355	360
Leu Gly Asp Ile Ser Tyr Pro Glu Met Val Thr Gly Phe Phe Phe Ile		365
	370	375
Leu Met Thr Val Leu Trp Phe Thr Arg Glu Pro Gly Phe Val Pro Gly		380
385	390	395
Trp Asp Ser Phe Phe Glu Lys Lys Gly Tyr Arg Thr Asp Ala Thr Val		400
	405	410
Ser Val Phe Leu Gly Phe Leu Leu Phe Leu Ile Pro Ala Lys Lys Pro		415
	420	425
Cys Phe Gly Lys Lys Asn Asp Gly Glu Asn Gln Glu His Ser Leu Gly		430
	435	440
Thr Glu Ser Ile Ile Thr Trp Lys Asp Phe Gln Lys Thr Met Pro Trp		445
	450	455
Glu Ile Val Ile Leu Val Gly Gly Gly Tyr Ala Leu Ala Ser Gly Ser		460
465	470	475
Lys Ser Ser Gly Leu Ser Thr Trp Ile Gly Asn Gln Met Leu Ser Leu		480
	485	490
		495

Ser Ser Leu Pro Pro Trp Ala Val Thr Leu Leu Ala Cys Ile Leu Val  
 500 505 510  
 Ser Ile Val Thr Glu Phe Val Ser Asn Pro Ala Thr Ile Thr Ile Phe  
 515 520 525  
 Leu Pro Ile Leu Cys Ser Leu Val Lys Ala Gly Leu Gly Val Asn Val  
 530 535 540  
 Ile Gly Leu Val Ile Val Met Val Ala Ile Asn Thr Trp Gly Val Ser  
 545 550 555 560  
 Leu Phe His Leu Asp Thr Tyr Pro Ala Trp Ala Arg Val Ser Asn Ile  
 565 570 575  
 Thr Asp Gln Ala  
 580

&lt;210&gt; 25

&lt;211&gt; 2019

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 25

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accaccacgg	cacatggaac	taagctagcc	caggctactca	ccacagtgga	cctcatctct	180
cttggcggtg	gcagctgtgt	gggcactggc	atgtatgtgg	tctctggcct	ggtggccaag	240
gaaatggcag	gacctgggtg	cattgtgtcc	ttcatcattg	cagccgtcgc	atccatatta	300
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&lt;210&gt; 26

&lt;211&gt; 672

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 26

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 20           25           30
Glu Ser Met Leu Glu Gly Thr Gly Thr Thr Thr Ala His Gly Thr Lys
 35           40           45
Leu Ala Gln Val Leu Thr Thr Val Asp Leu Ile Ser Leu Gly Val Gly
 50           55           60
Ser Cys Val Gly Thr Gly Met Tyr Val Val Ser Gly Leu Val Ala Lys
 65           70           75           80
Glu Met Ala Gly Pro Gly Val Ile Val Ser Phe Ile Ile Ala Ala Val
 85           90           95
Ala Ser Ile Leu Ser Gly Val Cys Tyr Ala Glu Phe Gly Val Arg Val
100           105           110
Pro Lys Thr Thr Gly Ser Ala Tyr Thr Tyr Ser Tyr Val Thr Val Gly
115           120           125
Glu Phe Val Ala Phe Phe Ile Gly Trp Asn Leu Ile Leu Glu Tyr Leu
130           135           140
Ile Gly Thr Ala Ala Gly Ala Ser Ala Leu Ser Ser Met Phe Asp Ser
145           150           155           160
Leu Ala Asn His Thr Ile Ser Arg Trp Met Ala Asp Ser Val Gly Thr
165           170           175
Leu Asn Gly Leu Gly Lys Gly Glu Glu Ser Tyr Pro Asp Leu Leu Ala
180           185           190
Leu Leu Ile Ala Val Ile Val Thr Ile Ile Val Ala Leu Gly Val Lys
195           200           205
Asn Ser Ile Gly Phe Asn Asn Val Leu Asn Val Leu Asn Leu Val Val
210           215           220
Trp Val Phe Ile Met Ile Ala Gly Leu Phe Phe Ile Asn Gly Lys Tyr
225           230           235           240
Trp Ala Glu Gly Gln Phe Leu Pro His Gly Trp Ser Gly Val Leu Gln
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Gly Ala Ala Thr Cys Phe Tyr Ala Phe Ile Gly Phe Asp Ile Ile Ala
260           265           270
Thr Thr Gly Glu Glu Ala Lys Asn Pro Asn Thr Ser Ile Pro Tyr Ala
275           280           285
Ile Thr Ala Ser Leu Val Ile Cys Leu Thr Ala Tyr Val Ser Val Ser
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Val Ile Leu Thr Leu Met Val Pro Tyr Tyr Thr Ile Asp Thr Glu Ser
305           310           315           320
Pro Leu Met Glu Met Phe Val Ala His Gly Phe Tyr Ala Ala Lys Phe
325           330           335
Val Val Ala Ile Gly Ser Val Ala Gly Leu Thr Val Ser Leu Leu Gly
340           345           350
Ser Leu Phe Pro Met Pro Arg Val Ile Tyr Ala Met Ala Gly Asp Gly
355           360           365
Leu Leu Phe Arg Phe Leu Ala His Val Ser Ser Tyr Thr Glu Thr Pro
370           375           380
Val Val Ala Cys Ile Val Ser Gly Phe Leu Ala Ala Leu Leu Ala Leu
385           390           395           400

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 Pro Glu Ser Asp Ile Asp Gly Phe Val Lys Phe Leu Ser Glu Glu His  
 435 440 445  
 Thr Lys Lys Lys Glu Gly Ile Leu Ala Asp Cys Glu Lys Glu Ala Cys  
 450 455 460  
 Ser Pro Val Ser Glu Gly Asp Glu Phe Ser Gly Pro Ala Thr Asn Thr  
 465 470 475 480  
 Cys Gly Ala Lys Asn Leu Pro Ser Leu Gly Asp Asn Glu Met Leu Ile  
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 Gly Lys Ser Asp Lys Ser Thr Tyr Asn Val Asn His Pro Asn Tyr Gly  
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 595 600 605  
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 610 615 620  
 Leu Pro Tyr Met Ala Pro Cys Leu Pro Phe Val Pro Ala Phe Ala Met  
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 Leu Val Asn Ile Tyr Leu Met Leu Lys Leu Ser Thr Ile Thr Trp Ile  
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&lt;210&gt; 27

&lt;211&gt; 2019

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 27

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&lt;210&gt; 28

&lt;211&gt; 672

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 28

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Met Ser Gly Phe Phe Thr Ser Leu Asp Pro Arg Arg Val Gln Trp Gly
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Ala Ala Trp Tyr Ala Met His Ser Arg Ile Leu Arg Thr Lys Pro Val
 20          25          30
Glu Ser Met Leu Glu Gly Thr Gly Thr Thr Thr Ala His Gly Thr Lys
 35          40          45
Leu Ala Gln Val Leu Thr Thr Val Asp Leu Ile Ser Leu Gly Val Gly
 50          55          60
Ser Cys Val Gly Thr Gly Met Tyr Val Val Ser Gly Leu Val Ala Lys
 65          70          75          80
Glu Met Ala Gly Pro Gly Val Ile Val Ser Phe Ile Ile Ala Ala Val
 85          90          95
Ala Ser Ile Leu Ser Gly Val Cys Tyr Ala Glu Phe Gly Val Arg Val
100          105          110
Pro Lys Thr Thr Gly Ser Ala Tyr Thr Tyr Ser Tyr Val Thr Val Gly
115          120          125
Glu Phe Val Ala Phe Phe Ile Gly Trp Asn Leu Ile Leu Glu Tyr Leu
130          135          140
Ile Gly Thr Ala Ala Gly Ala Ser Ala Leu Ser Ser Met Phe Asp Ser
145          150          155          160
Leu Ala Asn His Thr Ile Ser Arg Trp Met Ala Asp Ser Val Gly Thr
165          170          175
Leu Asn Gly Leu Gly Lys Gly Glu Glu Ser Tyr Pro Asp Leu Leu Ala
180          185          190
Leu Leu Ile Ala Val Ile Val Thr Ile Ile Val Ala Leu Gly Val Lys
195          200          205
Asn Ser Ile Gly Phe Asn Asn Val Leu Asn Val Leu Asn Leu Ala Val
210          215          220

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Trp	Val	Phe	Ile	Met	Ile	Ala	Gly	Leu	Phe	Phe	Ile	Asn	Gly	Lys	Tyr
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			245						250					255	
Gly	Ala	Ala	Thr	Cys	Phe	Tyr	Ala	Phe	Ile	Gly	Phe	Asp	Ile	Ile	Ala
			260					265					270		
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Ile	Thr	Ala	Ser	Leu	Val	Ile	Cys	Leu	Thr	Ala	Tyr	Val	Ser	Val	Ser
	290				295						300				
Val	Ile	Leu	Thr	Leu	Met	Val	Pro	Tyr	Tyr	Thr	Ile	Asp	Thr	Glu	Ser
305				310						315					320
Pro	Leu	Met	Glu	Met	Phe	Val	Ala	His	Gly	Phe	Tyr	Ala	Ala	Lys	Phe
			325						330					335	
Val	Val	Ala	Ile	Gly	Ser	Val	Ala	Gly	Leu	Thr	Val	Ser	Leu	Leu	Gly
		340						345					350		
Ser	Leu	Phe	Pro	Met	Pro	Arg	Val	Ile	Tyr	Ala	Met	Ala	Gly	Asp	Gly
	355						360					365			
Leu	Leu	Phe	Arg	Phe	Leu	Ala	His	Val	Ser	Ser	Tyr	Thr	Glu	Thr	Pro
	370				375						380				
Val	Val	Ala	Cys	Ile	Val	Ser	Gly	Phe	Leu	Ala	Ala	Leu	Leu	Ala	Leu
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Leu	Val	Ser	Leu	Arg	Asp	Leu	Ile	Glu	Met	Met	Ser	Ile	Gly	Thr	Leu
			405					410						415	
Leu	Ala	Tyr	Thr	Leu	Val	Ser	Val	Cys	Val	Leu	Leu	Leu	Arg	Tyr	Gln
		420						425					430		
Pro	Glu	Ser	Asp	Ile	Asp	Gly	Phe	Val	Lys	Phe	Leu	Ser	Glu	Glu	His
	435					440						445			
Thr	Lys	Lys	Lys	Glu	Gly	Ile	Leu	Ala	Asp	Cys	Glu	Lys	Glu	Ala	Cys
	450				455						460				
Ser	Pro	Val	Ser	Glu	Gly	Asp	Glu	Phe	Ser	Gly	Pro	Ala	Thr	Asn	Thr
465				470						475					480
Cys	Gly	Ala	Lys	Asn	Leu	Pro	Ser	Leu	Gly	Asp	Asn	Glu	Met	Leu	Ile
			485						490					495	
Gly	Lys	Ser	Asp	Lys	Ser	Thr	Tyr	Asn	Val	Asn	His	Pro	Asn	Tyr	Gly
		500						505					510		
Thr	Val	Asp	Met	Thr	Thr	Gly	Ile	Glu	Ala	Asp	Glu	Ser	Glu	Asn	Ile
	515					520							525		
Tyr	Leu	Ile	Lys	Leu	Lys	Lys	Leu	Ile	Gly	Pro	His	Tyr	Tyr	Thr	Met
	530					535					540				
Arg	Ile	Arg	Leu	Gly	Leu	Pro	Gly	Lys	Met	Asp	Arg	Pro	Thr	Ala	Ala
545					550					555					560
Thr	Gly	His	Thr	Val	Thr	Ile	Cys	Val	Leu	Leu	Leu	Phe	Ile	Leu	Met
			565					570						575	
Phe	Ile	Phe	Cys	Ser	Phe	Ile	Ile	Phe	Gly	Ser	Asp	Tyr	Ile	Ser	Glu
		580						585					590		
Gln	Ser	Trp	Trp	Ala	Ile	Leu	Leu	Val	Val	Leu	Met	Val	Leu	Leu	Ile
		595					600					605			
Ser	Thr	Leu	Val	Phe	Val	Ile	Leu	Gln	Gln	Pro	Glu	Asn	Pro	Lys	Lys
	610					615					620				
Leu	Pro	Tyr	Met	Ala	Pro	Cys	Leu	Pro	Phe	Val	Pro	Ala	Phe	Ala	Met
625				630						635					640
Leu	Val	Asn	Ile	Tyr	Leu	Met	Leu	Lys	Leu	Ser	Thr	Ile	Thr	Trp	Ile
			645						650					655	
Arg	Phe	Ala	Val	Trp	Cys	Phe	Val	Gly	Lys	Gln	Leu	Pro	Leu	Glu	Pro

660

665

670

<210> 29  
 <211> 777  
 <212> DNA  
 <213> Homo sapiens

&lt;400&gt; 29

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<210> 30  
 <211> 258  
 <212> PRT  
 <213> Homo sapiens

&lt;400&gt; 30

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Ala	Ala	Trp	Tyr	Ala	Met	His	Ser	Arg	Ile	Leu	Arg	Thr	Lys	Pro
		20					25					30		Val
Glu	Ser	Met	Leu	Glu	Gly	Thr	Gly	Thr	Thr	Ala	His	Gly	Thr	Lys
	35				40						45			
Leu	Ala	Gln	Val	Leu	Thr	Thr	Val	Asp	Leu	Ile	Ser	Leu	Gly	Val
	50				55					60				Gly
Ser	Cys	Val	Gly	Thr	Gly	Met	Tyr	Val	Val	Ser	Gly	Leu	Val	Ala
65				70					75					80
Glu	Met	Ala	Gly	Pro	Gly	Val	Ile	Val	Ser	Phe	Ile	Ile	Ala	Ala
		85						90					95	Val
Ala	Ser	Ile	Leu	Ser	Gly	Val	Cys	Tyr	Ala	Glu	Phe	Gly	Val	Arg
		100					105					110		Val
Pro	Lys	Thr	Thr	Gly	Ser	Ala	Tyr	Thr	Tyr	Ser	Tyr	Val	Thr	Val
	115					120						125		Gly
Glu	Phe	Val	Ala	Phe	Phe	Ile	Gly	Trp	Asn	Leu	Ile	Leu	Glu	Tyr
	130					135				140				Leu
Ile	Gly	Thr	Ala	Ala	Gly	Ala	Ser	Ala	Leu	Ser	Ser	Met	Phe	Asp
145				150					155					160
Leu	Ala	Asn	His	Thr	Ile	Ser	Arg	Trp	Met	Ala	Asp	Ser	Val	Gly
		165						170					175	Thr
Leu	Asn	Gly	Leu	Gly	Lys	Gly	Glu	Glu	Ser	Tyr	Pro	Asp	Leu	Leu
	180						185					190		Ala
Leu	Leu	Ile	Ala	Val	Ile	Val	Thr	Ile	Ile	Val	Ala	Leu	Gly	Val
	195					200					205			Lys
Asn	Ser	Ile	Gly	Phe	Asn	Asn	Val	Leu	Asn	Val	Leu	Asn	Leu	Val

210                      215                      220  
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<210> 31  
 <211> 777  
 <212> DNA  
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<210> 32  
 <211> 258  
 <212> PRT  
 <213> Homo sapiens

<400> 32  
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                          20                      25                      30  
 Glu Ser Met Leu Glu Gly Thr Gly Thr Thr Thr Ala His Gly Thr Lys  
                          35                      40                      45  
 Leu Ala Gln Val Leu Thr Thr Val Asp Leu Ile Ser Leu Gly Val Gly  
                          50                      55                      60  
 Ser Cys Val Gly Thr Gly Met Tyr Val Val Ser Gly Leu Val Ala Lys  
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 Glu Met Ala Gly Pro Gly Val Ile Val Ser Phe Ile Ile Ala Ala Val  
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 Ala Ser Ile Leu Ser Gly Val Cys Tyr Ala Glu Phe Gly Val Arg Val  
                          100                      105                      110  
 Pro Lys Thr Thr Gly Ser Ala Tyr Thr Tyr Ser Tyr Val Thr Val Gly  
                          115                      120                      125  
 Glu Phe Val Ala Phe Phe Ile Gly Trp Asn Leu Ile Leu Glu Tyr Leu  
                          130                      135                      140  
 Ile Gly Thr Ala Ala Gly Ala Ser Ala Leu Ser Ser Met Phe Asp Ser  
                          145                      150                      155                      160  
 Leu Ala Asn His Thr Ile Ser Arg Trp Met Ala Asp Ser Val Gly Thr

				165					170					175			
Leu	Asn	Gly	Leu	Gly	Lys	Gly	Glu	Glu	Ser	Tyr	Pro	Asp	Leu	Leu	Ala		
			180					185					190				
Leu	Leu	Ile	Ala	Val	Ile	Val	Thr	Ile	Ile	Val	Ala	Leu	Gly	Val	Lys		
		195					200					205					
Asn	Ser	Ile	Gly	Phe	Asn	Asn	Val	Leu	Asn	Val	Leu	Asn	Leu	Ala	Val		
	210					215					220						
Trp	Val	Phe	Ile	Met	Ile	Ala	Gly	Leu	Phe	Phe	Ile	Asn	Gly	Lys	Tyr		
225					230				235					240			
Trp	Ala	Glu	Gly	Gln	Phe	Leu	Pro	His	Gly	Trp	Ser	Gly	Lys	Pro	Ser		
				245					250					255			
Ser	Asn																